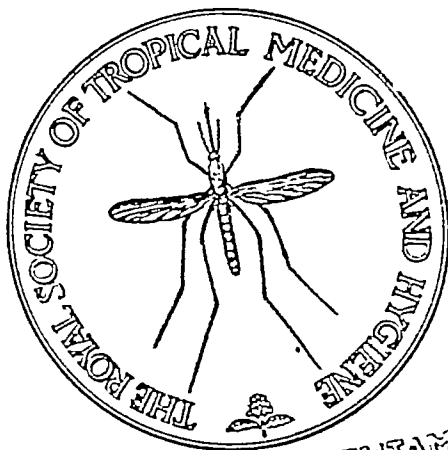




**TRANSACTIONS**  
OF THE  
**ROYAL SOCIETY OF TROPICAL  
MEDICINE AND HYGIENE.**

PATRON - HIS MAJESTY THE KING



**ZONAE TORRIDAE TUTAMEN**

**VOL 40 1946-1947.**

**London**  
**ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE**  
MANSON HOUSE, 26 PORTLAND PLACE, LONDON, W.1  
Telephone LANCASH 2127  
Telegrams ANGLIM LONDON

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**TRANSACTIONS**  
OF THE  
**ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE**

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**VOL 40 No 1 AUGUST, 1946**

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**LABORATORY MEETING**

of the Society held at

**The School of Tropical Medicine, Pembroke Place, Liverpool,**

on

**Thursday, 21st March, 1946, at 8 p m**

**C M WENYON, C M G , C B E , M B , B S C , F R S ,**  
**President, in the Chair**

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**DEMONSTRATIONS.**

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**THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE**

**DEPARTMENT OF TROPICAL MEDICINE**

**Prof B G Maegraith, Dr A R D Adams, Dr J D King, Dr D J Rigby,  
Dr R A Sladden, and Miss M M Tottey**

**Treatment of B T and M T malaria with paludrine (Temperature charts  
and diagrams illustrating plasma and urinary concentration of paludrine )**

Clinical cure of B T and M T malaria can be obtained with doses of paludrine ranging from 10 to 750 mg given twice daily for 14 days Over this range of dosage, paludrine has not given rise to toxic symptoms beyond occasional nausea and vomiting

Since June, 1945, cases of relapsing B T have been treated with either 50 or 500 mg paludrine twice daily for 14 days or with mepacrine, 200 mg four times daily for 2 days, followed by 100 mg three times a day for 10 days Courses of treatment have been administered to patients in rotation so that random sampling of subjects has been obtained Dosage has been checked by estimation of the drug in the plasma and urine

The results of this experiment are not yet complete, but relapses have occurred after all dosage regimes On these dosages, therefore, paludrine does not prevent relapses in established cases of B T

# LABORATORY MEETING

Relapsing and delayed primary attacks of B T are now being treated by giving single doses of paludrine ranging from 50 to 500 mg

In some cases the patients have been allowed to go on after a single dose until the disease has relapsed. Such relapses occur 3 to 8 weeks after the therapeutic dose

After treatment with a single dose of 300 mg patients are now receiving a dose of 100 mg once weekly for 6 months. So far as is known, relapses do not occur on this regime. Whether relapses occur after completion of the once weekly regimes is not yet known

Control experiments show that single doses of 400 mg mepacrine have similar curative effects on the acute attack of B T

Intravenous injection of 5 to 20 mg paludrine produces clinical cure in trophozoite induced therapeutic B T

Both primary and relapsing cases of M.T malaria respond to paludrine given orally. Doses of 50 to 600 mg twice daily for 14 days have proved successful. Cases are at present being treated with 100 to 200 mg twice daily and followed up for 6 months. So far on doses of 100 mg or more twice daily M.T malaria has not been found to relapse. Clinical cure has also resulted after 500 mg daily for 4 days.

Miss M M Tottay and Prof B G Macgrath.

## Pharmacology of paludrine in human subjects

Measurement of plasma paludrine concentration gives a fairly accurate estimate of red cell concentration

The following table shows the distribution of paludrine and mepacrine in whole blood plasma and packed red and white cells.

It will be seen that white cells contain about 120 times as much paludrine as red cells. (White cells fix about 3,400 times as much mepacrine as red cells)

Estimations of the drug were carried out in whole blood, plasma, packed red cells and leucocytes obtained from the same sample of blood.

The leucocytes were collected in washed centrifuge tubes, the volume of blood used being calculated from the haematocrit layer so that the buffy layer occupied the capillary stem.

	Paludrine $\mu\text{g/l}$ blood	Mepacrine $\mu\text{g/l}$ blood
Erythrocytes	831	28
Plasma	184	21
Leucocytes	125	159

	% Paludrine in whole blood	% Mepacrine in whole blood
Erythrocytes	73	13
Plasma	13.6	10
Leucocytes	13.4	77

Paludrine subject had been on a course of 500 mg b i d /14 days  
 Mepacrine subject had been on a course of 100 mg b i d /4 days

Figures were shown illustrating the plasma paludrine concentration after 50 and 500 mg twice daily for 14 days, and after single doses of 50, 100, 200, 300, 400 and 500 mg

After 50 and 500 mg twice daily for 14 days 30 to 50 per cent paludrine is excreted in the urine. After single doses ranging from 50 to 500 mg, up to 60 per cent is excreted in the urine.

#### Dr J. C Gage

##### The estimation of paludrine in urine

Paludrine reacts with copper in alkaline solutions to yield a complex which is soluble in benzene, the benzene solution when shaken with an aqueous solution of sodium diethyldithiocarbamate, develops a golden brown colour, the intensity of which is a measure of the amount of paludrine present. This reaction has been made the basis of a method of estimating paludrine in urine, it is not so sensitive as the method based on hydrolysis to *p*-chloro-aniline but it is adequate for the examination of urine of patients receiving paludrine therapy. The method is more simple to operate than the hydrolysis method and requires no elaborate apparatus, it is therefore suitable for clinical use in the field.

#### Dr A Spinks,

##### The pharmacology of paludrine in animals

The absorption, distribution and excretion of paludrine have been investigated in mouse, rat and rabbit. In all three species paludrine is rapidly absorbed, giving, however, only low concentrations in blood and plasma, of the order of 1 to 5 mg/l, following the oral administration of 80 mg/kg. The concentrations in blood are 2 to 4 times those in plasma, due to localization in cells, the concentrations in red cells being 4 to 8 times, and in white cells 10 to 100 times those in plasma. The leucocyte concentration, nevertheless, scarcely affects the blood/plasma ratio. Concentrations in tissues are

higher than in plasma, but vary greatly with the tissue, falling usually in the order lung, kidney spleen, liver heart, intestine, pancreas, muscle, fat, brain. The concentration in brain is minute and probably referable to the blood content of this organ that is, paludrine probably does not pass the blood brain barrier. The tissue/plasma concentration ratio rarely rises above 50 even in kidney and lung.

Paludrine is excreted mainly in the urine in which it is usually detectable for about 6 days following a single oral dose. Some drug is excreted in bile, and through the walls of the intestine, probably also in secretions other than bile, to give significant amounts in the faeces following intravenous administration. The total amount excreted in urine and faeces is usually less than 40 per cent. of that administered.

Paludrine shows marked qualitative similarity to mepacrine, suggesting that the biguanide side chain performs a "conductophoric function" (MAGIDSON), entirely analogous to that of the dialkylamino alkylamino side chain of mepacrine and allied drugs. In most respects the particular properties conferred on mepacrine by this side chain, which may be regarded as evidence of an affinity for cells shown at the expense of the body water are less markedly displayed by paludrine.

#### DEPARTMENT OF TROPICAL HYGIENE

Prof T H Davey

The method of measuring the area and diagonal of the aperture in standard and alternative mosquito netting. Specimens of alternative mosquito netting were on view showing various stages in the development of the netting finally issued by the Australian and British War Offices.

#### THE WARRINGTON YORKE DEPARTMENT OF CHEMOTHERAPY

Dr E M Lourie

A simplified medium for cultivation of leishmania: longevity of cultures.

The medium described below has been used for some years for routine maintenance of leishmania strains, as well as for primary culture in the diagnosis of kala-azar. It is semi-solid in consistency and comprises the following —

Ingredient	In the proportions of
Plain powdered agar ( <i>i.e.</i> not so-called ordinary or 2 per cent. nutrient agar)	0.3 gm.
Defibrinated rabbit blood	18 c.c.
Normal saline	100 c.

The proportions are quoted above in terms of the actual amounts frequently chosen, for convenience, in bulk-preparation of the medium, for distribution in a number of test-tubes

The medium is prepared as follows Agar and normal saline, in the required proportions, are placed in a flask and heated over a Bunsen burner to boiling-point The solution is then allowed to boil for about 2 minutes, after which the flask is placed in a pot of hot water, in which a thermometer is kept, and allowed to cool When the temperature registered by the thermometer has fallen to between 45° C and 50° C the flask is taken out, and the required volume of blood (previously obtained by cardiac puncture, and defibrinated by shaking with glass beads) is added and shaken to obtain an even distribution Before the medium has had time to cool to the point of setting, it is quickly poured into test tubes—3 to 15 c c per tube Strictly sterile apparatus and methods are of course essential, but it has been found unnecessary to autoclave any part of the medium

The tubes used for the cultures are of a standard test-tube size, about 15 cm long and 1.5 to 1.75 cm wide Cotton-wool plugs are not used, since it has been found quite satisfactory, in this climate, merely to cover the tubes with dome-shaped glass caps, about 3.5 cm deep and 2.5 cm wide, contaminations by air-borne bacteria, moulds, etc., have been negligible

*Advantages*—The medium is much simpler to prepare and to handle than the classical NNN medium There is no need to "seal" the tubes, or in any other way to promote and maintain "water of condensation," as in the use of NNN Dense growth occurs in the uppermost layers of the medium, from which flagellates are easily removed by means of a platinum loop, for examination or subcultivation In this respect it is as convenient to use as the media recommended by WENYON (1921), KLIGLER (1924), NOGUCHI and LINDENBERG (1925), and ADLER (1934), but it is of simpler composition than these preparations, in that plain agar is used instead of nutrient agar, and normal saline instead of more complicated solutions

The addition of glucose was tried, in order to determine whether richer or more long-lived cultures would result, but there seemed to be no advantage in this extra component

Tubes of medium may be put aside in the refrigerator for prolonged periods before use Several such tubes were sown as late as 14 months after preparation of the medium, and excellent cultures obtained

*Longevity of cultures*—Where cultivation is attempted for diagnostic purposes, the tubes should be incubated at 22° C to 25° C, since flagellates appear earlier at these than at lower temperatures For routine maintenance of strains, however, it is an advantage to keep the tubes at room temperature (varying between about 15° C and 21° C in the room where our cultures have been kept) Growth is slower under these conditions, flagellates first being seen a week or longer after sowing, but the individual subcultures last

a remarkably long time, provided there is sufficient medium initially in the tubes to compensate for loss by evaporation.

The very slight amount of attention demanded in routine maintenance of strains under these conditions is shown by the protocols below —

*Leishmania tropica.*

Subculture No.	Date of subculture.	Time since last subculture	
19	20th Aug., 1936		11 subcultures during 3 years and 7½ months
20	18th Oct.,	2 months	
21	31st Dec.,	2	
22	23rd Mar. 1938	3	
23	9th June,	2½	
24	14th Aug.	2	
25	1st Nov.	2½	
26	7th Jan., 1937	2	
27	19th Dec.,	11	
28	31st Oct., 1938	10½	
29	12th Feb., 1939	3½	
30	27th May	3½	

*Leishmania donovani*

PRIMARY CULTURE FROM STERIL FINGERPRINT FLUID BOWN 18TH OCT. 1937

Subculture No.	Date of subculture.	Time since last subculture	
1	12th Dec., 1937	3 months (since primary culture)	4 subcultures during 1 year and 7 months
2	21st Oct., 1938	10 months	
3	12th Feb., 1939	2½ months	
4	27th May		

As shown by these data, successive subcultures have often been made at intervals of 2 or 3 months. However many of these cultures when examined 4 to 6 months or more after sowing have still been found to contain moving flagellates. In several instances, when the amount of medium initially in the tube was about 15 c.c. individual subcultures remained viable for as long as 10 and 11 months, far outstripping the periods of some 110 days to which attention was drawn by Row (1935)

Recently prepared culture tubes were demonstrated, together with two tubes in which subcultures of *L. tropica* had been maintained for many months.

On the latter tubes the level of the medium was marked at intervals, to show the amount of loss by evaporation, as summarized in the table below —

Tube (15 cm × 1 7 cm)	Tube-length occupied by medium		
1	12th Dec, 1937 (start)		7½ cm
	5th May, 1938 (4½ months from start)	Still + ve	5½ cm
	29th Oct, „ (10½ months from start)	— ve	4 cm
2	2nd June, 1937 (start)		11 cm
	21st Oct, „ (4½ months from start)	Still + ve	9 cm
	17th Feb, 1938 (8½ months from start)	Still + ve	7½ cm

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Dr E M Lourie, Dr A O F Ross and Mr J Williamson

Therapeutic action of crystalline penicillin on spirochaetoses

(a) Table comparing effects of (i) partially purified penicillin, (ii) crystalline penicillin II, and (iii) crystalline penicillin III on *Spirochaeta recurrentis* infections in mice (Details to be published in the *British Medical Journal*)

(b) Colour photographs of primary and secondary syphilis before and after treatment by penicillin II

Dr H O J Collier and Dr E M Lourie

Table showing relationship of chemical constitution to leishmanicidal activity *in vitro*, among aromatic diamidines and related compounds (Details to be published in the *Annals of Tropical Medicine and Parasitology*)

## DEPARTMENT OF ENTOMOLOGY AND PARASITOLOGY

Miss M A Hill

The life-cycle and habits of British *Culicoides*

Specimens showing the life history and graphs illustrating the seasonal abundance of *Culicoides* sp were shown

In strains reared at room temperature the egg stage of *C. obsoletus*, Mg



lasts from 30 hours to 11 days and the larval stage, 5 months. The eggs of *C. impunctatus* Goet. hatched in 14 days, and the larval stage lasted 7 months. From eggs of *C. obsoletus* laid in May and kept in the open, adults were reared in October but larvae which hatched from eggs of *C. impunctatus* laid at the same time, and also kept in the open, had not pupated after 10 months. In 1945 *C. impunctatus* was first collected at Knowsley Park, Liverpool, at the beginning of April. There was a steady rise in its abundance until the beginning of June, when the species reached its peak. No specimens were caught after 5th August. The first specimens of *C. obsoletus* were also collected at the beginning of April, and the species reached a peak of abundance during the end of May and the beginning of June. They were found throughout the summer and another peak of abundance was recorded at the end of September and the beginning of October.

The numbers of larvae recovered from a selected site dropped from a level which was steady during March and April, 1945 and were least abundant during May. In June and July there was a sharp rise in abundance of larvae, and in July and August the numbers reached a peak. Throughout the winter the numbers dropped steadily to a level, which in March, 1946 was similar to the level in March, 1945. From this site pupae were recovered during April, May and June, and *C. impunctatus* emerged.

Mr K Unsworth and Prof R M Gordon

Sections of rabbit skin showing attached *Trombicula* larvae

The sections showed the mouthparts of mites penetrating the stratum corneum, and the extraordinary sucking tube extending into the corium and formed as a result of the reaction by the host tissues and the secretions of the mite. The sections demonstrated that this curious method of feeding causes an area of hyaline degeneration in the corium which usually takes the form of a sharply defined column of degeneration vertical to the skin.

Mr K Unsworth and Prof R M Gordon

A technique for maintaining *Phlebotomus papatasi* in this country

Two techniques were demonstrated —

(a) A modification of that used by SHORR *et al.* (1926) in which the flies were fed on the arm of a human volunteer. By this method a strain of *P. papatasi* has been maintained at Liverpool for 5 months.

(b) This technique was time-consuming and various attempts were therefore made to find some method of breeding the flies which would dispense with the use of human volunteers for blood meals, and allow of easy access to the breeding chambers.

The most promising method so far adopted is one in which a high humidity is attained within a chamber constructed of Keene's cement. The food for the

larvae is spread on a porous container resting on the floor of the chamber. Baby rats are introduced daily and serve as a source of blood for the flies.

The results so far obtained by this method have been promising, but the apparatus is still in the experimental stage.

Prof R M Gordon, Mr K Unsworth, Miss M A Hill and Dr D S Bertram

Investigations on *Liponyssus bacoti* as a vector of *Litomosoides carini* in cotton rats

### 1 *Method of isolation of the mites*

(a) From bedding The bedding material is spread inside a tube 3 feet long and 1 inch in diameter, the tube being suspended on terminal brackets. An electrically heated coil pulled by mechanical traction slowly traverses the length of the tube, driving the mites before it into a collecting chamber.

(b) From the nests of *R. rattus* The nest is teased out into a large transparent funnel leading to a darkened chamber at the lower limit of the stem of the funnel. A 60-watt lamp hangs above the funnel and, provided the material is well dispersed, a high proportion of mites collect in the dark chamber.

### 2 *The life-cycle of L. bacoti*

Exhibits of the life-cycle of *L. bacoti* showing the eggs, the relatively immobile, non-blood-sucking larvae, larval casts, unfed protonymphs and adult male and female mites.

### 3 *Method of maintaining strains of L. bacoti*

Apparatus was shown which is being used and developed for the maintenance of strains of *L. bacoti* and for the transmission of *L. carini* by the mite to the vertebrate host.

### 4 *The development of L. carini in L. bacoti*

Various stages in development were shown and, since the demonstration, the infective form has been recovered. These results confirm those of WILLIAMS and BROWN (1945).

### 5 *Transmission of L. carini to the uninfected cotton rat and to the white rat*

The demonstration showed the method of maintaining colonies of *L. bacoti* on the cotton rats, the colonies being isolated by means of water surrounds. In this way a number of cotton rats and white rats have been exposed to infection with the filaria. Sufficient time has not yet elapsed for the appearance of microfilariae in the peripheral blood. That the white rat is subject to infection with the same species of filaria, *L. carini*, as the cotton rat, has been proved by

CHANDLER (1931) who states "*L. signodontis*\* occurs in a high percentage of cotton rats in the vicinity of Houston and was found also in a white rat born and raised in the Rice Institute animal house, where some infected cotton rats were kept.

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 WILLIAMS, R. H. & BROWN H. W. (1945). *Science* 102 (2634) 432.

Prof R M Gordon Miss M A Hill and Dr D S Bertram.

Technique employed for obtaining bacteria-free suspensions of sporozoites of *Plasmodium gallinaceum*.

A batch of *Aedes aegypti* are fed 18 days prior to the experiment, on a fowl infected with *P. gallinaceum*, and showing numerous gametocytes in the peripheral blood.

One lightly anaesthetized mosquito is held by the proboscis in forceps under a stream of rapidly falling drops of 75 per cent. alcohol for 1½ minutes. After this preliminary washing in alcohol the mosquito is rapidly drained on two successive slips of sterile filter paper and transferred to a drop of sterile Tyrode's solution on a sterile slide placed under a dissecting microscope. The mosquito is then dissected with sterile needles and the salivary glands removed without cutting the oesophagus. The dissected glands are next transferred to a fresh drop of sterile Tyrode's solution and finely minced. A rigid aseptic technique, such as that employed in tissue culture, is observed throughout all these manipulations and the subsequent inoculation of media and tissue.

If sporozoites are found to be present and motile, one platinum loopful of the emulsion is sown in 10 c.c. of Brewer's medium.† A sample of the sporozoite emulsion is then injected into a fowl, in order to confirm that the sporozoites have not been injured by the sterilizing alcohol treatment of the mosquito.

The results suggest that a high proportion of the glands obtained by this technique are free of viable bacteria and fungi and that the treatment has no injurious effect on the sporozoites they contain.

Dr D S Bertram and Prof R M Gordon

Apparatus used for breeding out adult *Simulium* from collected larvae.

The control of *Simulium* breeding places is notoriously difficult and the testing of larvicidal substances in the laboratory has proved unsatisfactory

Synonym of *L. cervicali*.

† The sodium thioglycollate medium recommended by BARBER (1940), and commonly used by the Blood Bank for testing the sterility of plasma, consists of —

	Per cent.		Per cent.
Pork infusion solids	1	Agar	0.05
Peptone (thio)	1	Glucose	0.1
Sodium chloride	0.5	Methylene blue	0.002
Sodium thioglycollate	0.1		

since it is difficult to imitate the natural environment of the larvae in fast-running, highly oxygenated water

The apparatus demonstrated consisted of a cement block designed to provide a wedge-shaped depression on a slope of about 45° leading into concavities 4 inches to 5 inches deep in the base of the block. The cement block rested in a wooden case with glass sides above the level of the cement, and a gauze top through which the aerating device projected.

The principle of the apparatus is that a continuous flow of water, oxygenated by passage through a glass filter pump, passes down the sloping groove and through the concavities or pools to a metal outflow pipe discharging into a sink.

This technique was used with success in West Africa by one of the exhibitors to rear certain species of *Simulium* (but not *S. damnosum*) from egg to adult. In Africa the piped water supply was direct from a natural reservoir, and although it is not certain that the Liverpool water supply, which is at present chlorinated, will prove equally suitable, full grown larvae collected in North Wales have pupated and produced adults after 12 days of exposure to a constant aerated stream of this water supply.

Mr K Unsworth

Skin sections showing *Demodex canis*

A skin section, taken from a case of follicular mange in the dog and showing the mite *Demodex canis*, together with its ova, in the hair follicles.

Mr K Unsworth

Plerocercoid of *Diphyllobothrium* sp from the stickleback, *Gasterosteus aculeatus*

A plerocercoid of *Diphyllobothrium dendriticum* recovered from the stickleback (*G. aculeatus*) 6 weeks after exposure to infection with procercoids. It was suggested that these small fish may form a reservoir for the final fish host of *Diphyllobothrium*.

Pike became infected with plerocercoids when fed on infected sticklebacks, and it was suggested that similar small fish might play a part in the life-cycle of other species of *Diphyllobothrium* forming a reservoir of infection for the final fish host.

Sections prepared from material supplied by Dr BHASKARA MENON, showing the distribution of adult and larval forms of *Conspicuum flavescens* in the abdomen of the lizard *Calotes versicolor*.

Dr BHASKARA MENON and his colleagues\* have shown that infection with this worm has been known to produce elephantiasis in the Indian garden lizard.

\* MENON, T BHASKARA, RAMAMURTI, B & SUNDARASIVA RAO, D (1944) *Trans R Soc Trop Med Hyg*, 37, 373

(*C. oervecolor*), and have published a full account of the life-cycle of the worm, including its development in *Culex fatigans* and the pathological lesions it produces in the reptile host.

Lave specimens of infected lizards were kindly sent to the Liverpool School by Dr MEXON. The sections shown were made from one of the animals which died shortly after arrival, and illustrate Dr MEXON's description of the appearances produced by the parent worms when lying inside lymphatic vessels in the mesentery.

#### Sir Philip Manson-Bahr

A series of photographs from Quito, Ecuador (8,850 metres) taken by Prof.

A Leon, Professor of Tropical Medicine, Central University, Quito.

The pictures represented pinta, espundia, verrugo peruana, and mycetozoa, as seen in Ecuador.

#### Dr George Macdonald.

Pictures of *Anopheles gambiae* in Egypt.

The exhibit illustrated by means of maps and photographs the location and dates of severe epidemics of malaria in the Nile Valley which were caused by *Anopheles gambiae*. This mosquito had not been previously recorded in Egypt. Additional photographs showed the methods of control used by the Gambiae Eradication Service under the supervision of the Rockefeller Health Foundation.

#### Mr W. R. Jones.

Experimental amoebiasis: chemotherapeutic studies using experimentally infected rats.

An experimental infection with *Entamoeba histolytica*, may be produced in young rats. It is suitable for use in chemotherapeutic studies. The test drug is administered 24 hours after the infection, and an assessment is made of the effect by a postmortem examination made after 6 days. The therapeutic effect is measured by the use of a statistical method in which the average degree of infection of a treated group is compared with that of a control group.

The following items were demonstrated.

1. A medium for the large-scale cultivation of *Entamoeba histolytica*.
2. Amoebic ulceration in the caecum of experimentally infected rats.
3. Slides and photographs showing superficial and extensive amoebic ulceration in the caecum, colon and ileum of rats.
4. Charts illustrating the normal course of the infection.
5. Charts demonstrating the therapeutic effect of emetine, chiniofon, carbarsone and stovarsol.





## ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place,

on

Thursday, 16th May, 1946, at 8 p m

THE PRESIDENT

C M WENYON, C M G , C B E , M B , B S C , F R S  
in the Chair

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## PAPER.

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### OBSERVATIONS ON TSUTSUGAMUSHI DISEASE (SCRUB TYPHUS) IN ASSAM AND BURMA

#### PRELIMINARY REPORT

BY

THOMAS T MACKIE, COLONEL, M C , AUS, *et al* \*

*Executive Officer, The United States of America Typhus Commission, India Burma  
Theatre*

*(From the China-Burma-India Field Headquarters, United States of America Typhus  
Commission, War Department, Washington, 25, D C , U S A )*

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#### INTRODUCTION AND ACKNOWLEDGMENTS

The observations reported in this paper constitute a preliminary report of investigations on tsutsugamushi disease (mite-borne typhus, scrub typhus)

\* The following co-authors were members of the Field Headquarters, United States of America Typhus Commission, War Department, Washington, 25, D C —

GORDON E DAVIS, Major, Sn C , AUS , HENRY S FULLER, Captain, M C , AUS , JOHN A KNAPP, Captain, M C , AUS , MAURICE L STEINACKER, Captain, Ord , AUS , KENNETH E STAGER, Captain, Inf , AUS , ROBERT TRAUB, Captain, Sn C , AUS , WILLIAM L JELLISON, Sanitarian (R ) , USPHS , DICK D MILLSAUGH, 1st Lieutenant, Sn C , AUS , ROBERT C AUSTRIAN, Captain, M C , AUS , E JOHN BELL, Captain, Sn C , AUS , GLEN M KOHLS, Major, Sn C , AUS , WEI HSI, M D National Epidemic Prevention Bureau, Kunming, China , and JAMES A V GRESHAM, Captain, Kachin Rifles



conducted by the United States of America Typhus Commission in the China-Burma-India theatre from December 1944 to November 1945. These studies for the most part were carried out in the vicinity of Ledo, Assam, and in the region about Myitkyina, Upper Burma. The successful outcome of the investigations was greatly facilitated by the support and co-operation of many individuals and many organizations.

The first detailed scientific study of tsutsugamushi disease in Japan defined the clinical syndrome and described the principal features of the epidemiology. It was observed to have a strict seasonal incidence, occurring in the spring and early summer in river valleys subject to inundation, appearing as the flood waters receded and agricultural workers returned to the land. The incidence was restricted to individuals working on the flooded areas and coincided with the appearance of large numbers of mites which attached to man and to the vole, *Microtus montebellii* Milne-Edwards. The mites were believed to play a part in the aetiology of the disease and it was suggested that an infectious agent was introduced into man by the mite subsequently described as *Trombicula akamushi* (BRUMPT 1910 TANAKA, 1899). It was shown that the disease did not occur in areas where this mite was not found, and that there was no seasonal correlation between the incidence of the disease and the appearance of other species of mites. It was likewise observed that *T. akamushi* did not attach to two hosts successively leaving the initial host after completing its engorgement and carrying on the remainder of its life cycle in the soil.

Early experimental studies in Japan appeared to confirm the belief that the mite functioned as a vector of the disease. Larvae of *T. akamushi* reared from adults held in the laboratory were allowed to attach and to feed upon a Japanese monkey which subsequently developed a febrile condition believed to be the homologue of the disease in man. (MIYAJIMA and OKUMURA, 1917) It was further shown that monkeys and other experimental animals exposed

While it is impossible to make specific acknowledgment to all, we wish to express our particular appreciation to the following American and British civilian and military authorities —

The theatre surgeons, China-Burma-India Theatre, Brigadier General JAMES E. BAYLES, U.S.A., Colonel ROBERT P. WILLIAMS, M.C. and Colonel HART R. LUNDENBERG, M.C. Brigadier General JOHN P. WILLEY, U.S.A., Commanding General, Mairi Task Force. Colonel R. A. HUNSFIELD, Corps of Engineers, Commanding Officer, Southern Area Command; Colonel VERNON W. PETERSON, M.C. Surgeon, Northern Combat Area Command; Lieut.-Colonel W. W. HIRSH, M.C., Surgeon, Mairi Task Force and Captain ROY MELVIN, Sanitary Corps, who was in charge of the Mite Laboratory during the period from October 1944 to August, 1945.

Particular thanks are due to the Commanding Officers and the staffs of the following Medical Department installations for their constant and unfailing assistance and co-operation: the 20th General Hospital, the 14th Evacuation Hospital, the 48th Evacuation Hospital, the 73rd Evacuation Hospital, the 25th Field Hospital; the 44th Field Hospital and the 9th Medical Laboratory.

Dr R. LAWTHWAITE, Medical Research Council of Great Britain, and Lieut.-Colonel J. R. ADY, R.A.M.C., Scrub Typhus Laboratory, Imperial, India Command. Colonel WILKIE, Senior Civil Affairs Officer, Myitkyina, Burma, the Commanding Officer, 94th Indian General Hospital. Dr S. R. SARVOOD, the Haffkine Institute for Medical Research and the Calcutta School of Tropical Medicine.

in the endemic areas became infested with mites and that certain developed "typical" illnesses, in some instances with the development of primary lesions or eschars, similar to those observed in man. Payments indicated that an infectious agent was present in the blood during the febrile period, disappearing when the temperature returned to normal. That the infection could be transmitted from man to monkey by blood, especially when the blood was taken about the time of appearance of the eruption. Recovered monkeys were refractory to re-inoculation. (KITASHIMA and MIYAJIMA, 1918) More direct experiments incriminated the mites. Suspensions of adult *T. akamushi* collected and of larvae reared in the laboratory, when injected into monkeys were to be capable of producing "typical" infection in them. (NAGAYO 1921) When laboratory reared larvae were allowed to attach to one of the animals subsequently developed a febrile condition which considered to be characteristic and which was associated with a primary lesion at the site of the attachment. Blood from this monkey was infected and following recovery, the animal was refractory to re-inoculation. (KAWAMURA, 1926) The aetiological agent, however, was not described. 1924 when diplococcus-like and short rod forms staining by Giemsa's method and by azur II were observed in smear preparations and sections of the primary lesions and other infected human and animal tissues. (NAGAYO *et al*, 1924) The name *Rickettsia orientalis* was subsequently given to this organism. (NAGAYO *et al*, 1930), and is considered by some to have the strongest basis to recognition. (PHILIP, 1943)

The basic studies of the Japanese workers were completed by the recovery of the infectious agent from the spleen of naturally infected voles, *Microtus montebellii* Milne-Edwards. (KAWAMURA and IMAGAWA, 1931)

Meanwhile investigations were proceeding into the typhus-like diseases encountered in the Dutch East Indies and Malaya. One of these, the "pseudotyphoid" of Deli in Sumatra, was early thought to be a variety of the tsutsugamushi disease of Japan and was suspected to be transmitted to man by a mite. (SCHUFFNER, 1915) This mite was subsequently described as *Trombicula deliensis* by WALCH in 1923 and was recognized to be closely related to *T. akamushi* of Japan. (WALCH, 1923)

An epidemiological study of an outbreak of "pseudotyphoid" in Sumatra yielded important confirmatory evidence concerning the role of the mite *T. deliensis*. A direct correlation was found between the prevalence of this species of mite and the hazard of infection. It was noted that the disease occurred primarily among labourers engaged in clearing brush and "alang" grass from land that had been overgrown during disuse and that these labourers were often infested with *T. deliensis*. Workers in primitive forest, on the other hand, were not attacked by this mite and the disease did not occur among them. Following removal of an attached mite, a primary lesion was observed to develop in one instance at the exact site of attachment.

ment. This species of mite was found to infest wild rats. Injection of a suspension of larvae into a gibbon produced a febrile disease which was considered significant. (WALCH and KEUKENSCHRIJVER, 1925)

It was recognized that tsutsugamushi disease was present in some parts of Malaya and that the epidemiology was similar to that in Sumatra. Large numbers of *T. deliensis* were found infesting the wild rats in areas where the disease occurred. (FLETCHER, LESSLAR and LEWTHWAITE, 1928.) Other typhus-like diseases not presenting an eschar were classified as urban, and rural or "scrub" typhus. Shortly thereafter it was shown that these could be differentiated by serological methods. Sera from cases of the urban form agglutinated *Proteus* OX19 but gave no reaction with *Proteus* OXK. Sera from the rural or scrub disease, on the other hand, yielded a negative reaction with *Proteus* OX19 and a positive reaction with the Kingsbury strain. (FLETCHER, LESSLAR and LEWTHWAITE, 1929) This observation established the identity of tsutsugamushi disease and rural or "scrub" typhus, and demonstrated that the clinical distinction based on the presence or absence of eschar was invalid.

Further investigations of the rural or scrub form of tropical typhus led to the isolation of two strains of rickettsiae from wild rats trapped in an endemic area. These strains exhibited cross immunity with human strains. The recovery of the infectious agent from the rodents was considered to be conclusive evidence that the wild "brown" rat was the reservoir for the causative agent and that the larval mite *T. deliensis* was almost certainly the vector of disease. (LEWTHWAITE and SAVOOR, 1936) Unfortunately no specific identification of the rat was made, and no experimental proof of the presence of the causative agent in the mites was presented. Subsequently the conclusion was reached that tsutsugamushi disease in Malaya and the rural form of tropical typhus, "scrub typhus," were caused by the same aetiological agent, *R. orientalis*. It was further established that the causative agent of Malayan tsutsugamushi and scrub typhus, and of Sumatran mite fever "or "scrub typhus" were identical and gave cross protection in experimental animals. (LEWTHWAITE and SAVOOR, 1940 KOUWENHAAR and WOLFF 1942.)

While the terms tropical typhus and tick typhus appear frequently in the medical literature of India, it was not possible to distinguish between the specific disease entities prior to the introduction of the Weil Felix reaction using standardized suspensions of the "O" antigen of the three strains of *Proteus* XK, X19 X2. Following the adoption of this diagnostic technique in 1934 reports began to appear of scattered cases of a typhus-like disease which gave significant agglutinin titres against the OXK strain of *Proteus*. These were recorded first from the Simla Hills (MACNAMARA, 1935), and shortly thereafter from Lahore, Meerut, Bengal and Assam, Deccan, and one case from Burma. (BOYD 1933) Confirmation of the presence of OXK positive typhus in the Simla Hills was shortly provided (BUSH, 1936 COVILL, 1936), and it was noted that in this region the disease occurred as sporadic

cases or focal outbreaks in rural or semi-rural areas, appearing especially immediately after the rainy season during August, September and October. As the Weil-Felix reaction was more widely applied, it became apparent that the disease was distributed over a wide area although its incidence was not great in any region. Thus, positive sera were recorded from various parts of Assam (WOODHEAD and DUTTA, 1941), from Bombay (PATEL, 1943), Ceylon (WIJERAMA, 1938, NICHOLLS, 1940), and the Madras Presidency (King Institute, 1938).

Extensive serological studies reported in 1936 indicated a widespread distribution in Burma. Positive sera were obtained in Upper Burma from Yamethin, Meiktila, Kyaukse, Shwebo, Chin Hills, Katha, Southern Shan States, Northern Shan States, Mandalay, Maymyo, and Myitkyina, and in South Burma from Rangoon, Syriam, Henzada, Prome, Toungoo, and Bassein (MAITRA and GUPTA, 1936).

Five species of larval trombiculid mites were identified in the Simla Hills region of which only two were considered of potential importance, *Trombicula deliensis* and *Trombicula acuscutellaris*. *T. deliensis* was found to infest wild rats throughout the area and to be particularly abundant during the season when cases of the XK type of the disease were occurring. This mite therefore was regarded as the probable vector of scrub typhus in that region although no experimental evidence was put forward in support of the suspicion (MEHTA, 1937).

The few attempts which were made to isolate the rickettsiae from wild rats were unsuccessful (SMITH and MEHTA, 1937). Other investigations of a possible rodent reservoir of the rickettsiae were based upon the Weil-Felix reaction in rodent sera. Titres of OXK agglutinins from 1/25 to 1/200 were obtained with sera from squirrels in Madras (King Institute, 1937) and similarly sera from wild rats in the Simla Hills region showed titres of 1/125 or greater. The significance of the serological findings in rodents, however, is open to serious doubt. It has been shown in similar studies of endemic (murine) typhus that the Weil-Felix reaction with rat sera may give both false positive and false negative reactions (BRIGHAM and BENGSTON, 1945). Both H and O variants of *Proteus*, and one strain culturally and serologically identical with OXK have been recovered from the blood of wild rats (VAUCEL and BRUNEAU, 1937). A positive Weil-Felix reaction, therefore, may be the expression of this infection rather than of infection by the rickettsiae. It has been pointed out, moreover, that rats commonly harbour *Spirillum minus*, which of itself produces a positive reaction with *Proteus* OXK (SAVOOR and LEWTHWAITE, 1941).

Interpretation of the incidence and distribution of scrub typhus in India and Burma before the war is still further complicated. Many of the cases presenting positive serological evidence have been clinically atypical. In some no rash was present and in numerous others there was no eschar or primary lesion. The report that significant titres against OXK may be observed in

relapsing fever (ELSDON-DREW 1943) suggests that certain of these early cases may have been misinterpreted because of a non-specific Weil-Felix reaction.

Knowledge of the disease at the outbreak of the war with Japan (in 1941), may be briefly summarized. It was recognized to occur in Japan, the Philippine Islands, Indo-China, Formosa, Australia, New Guinea, Papua, Java, Sumatra, Malaya, Ceylon, and various parts of India and in Burma. It was variously known as trugamushi disease, tropical scrub typhus, pseudotyphoid of Deli Sumatran mite fever and Mosman fever. In Japan and in the Simla Hills region of India the disease had a definite seasonal incidence related to the rains and to the appearance of trombiculid mites. *T. akamushi* had been shown to be a host for the rickettsiae and incriminated as the vector in Japan on both epidemiological and experimental grounds. A closely related species, *T. deliensis* was considered to be the vector in Sumatra, Malaya, and the Simla Hills of India. The evidence supporting this belief, however was epidemiological and the mites had not been shown to harbour the infectious agent.

In Japan the vole *Aficrotus montbellus* was known to be a host for *Rickettsia orientalis* and these organisms had been recovered from wild "brown" rats in Malaya. These observations are the basis for the postulate that certain rodents serve as reservoirs of the infection.

In the course of World War II scrub typhus became a disease of considerable military importance in India, South Eastern Asia and New Guinea. Extensive investigations undertaken in the last area by the United States of America Typhus Commission have already been reported. (BLAKE, MAXCY SADUSK, KOHL and BILL, 1945 KOHL, ARMSTRONG IRONS and PHILIP 1945 PHILIP and KOHL 1945) In 1943 an extensive outbreak occurred among troops undergoing jungle training in Ceylon. As the forces were mobilized along the Burma border in preparation for the offensive operations the incidence among military units reached serious proportions. In the following year an unidentified febrile disease resembling typhus made its appearance among United States and Chinese army units in the vicinity of Ledo, Assam. At first termed "CBI Fever" it was shortly recognized to be scrub typhus or trugamushi disease. (PEPPER, 1944.) In late 1943 the disease was recognized to occur along the greater part of the front from Fort Hertz in the north through Assam, Imphal and south to Cox's Bazar. It constituted one of the serious medical problems of the Wingate Raiders, the Chinese troops undergoing training in the vicinity of Ledo, Assam, and the Merrill Marauders in the campaign through the Hukawng Valley to Myitkyna. This sudden translation into a disease of considerable military importance in the theatre led to a request by the Commanding General, China-Burma-India theatre that the United States of America Typhus Commission undertake investigations into the epidemiology and make recommendations for the protection of military personnel. The Field Headquarters of the Commission arrived in the theatre in October 1944 and set up its station in Myitkyna, Burma, where it remained throughout the period of the studies. [The first report of these investigations has recently been published (MAXCY *et al.*, 1946).]

The individual case records of all U S Army Hospitals reporting cases of scrub typhus were studied to determine the incidence of the disease, the seasonal distribution of cases, and the probable sites at which the infection was acquired. All questionable diagnoses were eliminated. Unfortunately, the records of 300 Chinese patients treated from November, 1943, to November, 1944, had been destroyed before this study was undertaken. The total number of cases included is, therefore, considerably below the actual number which occurred. With this exception the data presented are considered to be accurate within a very small margin of error. From 1st November, 1943, to 1st September, 1945, a total of 1,098 cases of scrub typhus occurred among United States and Chinese troops. The overall case fatality rate was 8.9 per cent.

Since adequate protective measures, including the use of dimethyl phthalate, were not used by troops until the spring of 1945, the incidence rates and the geographical distribution of the cases present essentially an unmodified picture of the epidemiology in a heavily exposed, non-immune and completely unprotected population.

The date of the onset of symptoms was recorded in each case and by applying an arbitrary incubation period of 10 days the approximate date of infection was determined. The position of the individual's unit and its mission on this date were obtained from the Weekly Situation Reports of the Northern Combat Area Command (N C A C). The locations of service troops not included in these reports could be determined from the hospital records or from the records of the various Headquarters, Service of Supply. While such data obviously cannot give the exact locations at which the infection was acquired, they do afford a dependable indication of the geographical distribution of the disease.

With few exceptions the disease is not known to have occurred in United States or Chinese troops outside of Burma or the Ledo area of Assam. The cases originated in varying numbers throughout the terrain traversed by the Stilwell Road from Ledo through Shungbwyang, Shaduzup, Mogaung, Myitkyina, Bhamo, Namkham, and south to the limit of the penetration of the combat forces south of Wanting. Cases likewise occurred in the Fort Hertz region, the mountains east of Shaduzup between that point and the Irrawaddy Valley, along the narrow gauge railway south of Mogaung, and in the country to the south of Bhamo and west of Namkham. In this wide and general distribution certain areas stand out as the apparent source of unusually large numbers of cases. Such areas are the region immediately to the east of Ledo, the vicinity of Shungbwyang, the mountainous region east of Shaduzup, the Myitkyina area, the country about Mogaung, and the areas to the south and west of Namkham. It is impossible to state whether the variation in incidence represents different levels of endemicity. It is evidence, however, that scrub typhus is widely distributed throughout the entire region in which United States and Chinese troops were operating in Burma (Fig 1, p 22 *et seq*).

FIG. 1. PART I.

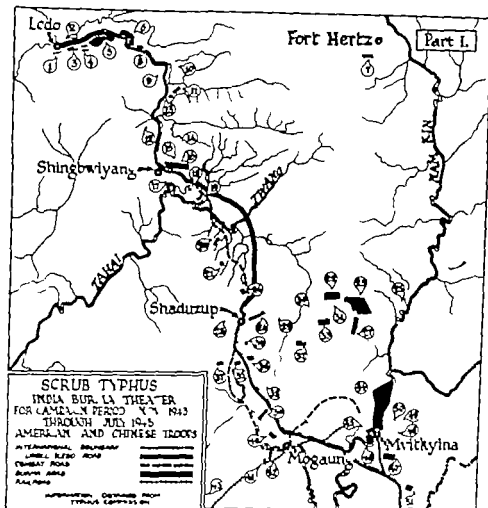


FIG. 1. Parts I and II.—SCRUB TYPHUS DISTRIBUTION OF PROBABLE DEFECTIVE AREAS. Numerals refer to sectional map references. Size of shaded areas roughly proportional to number of cases.

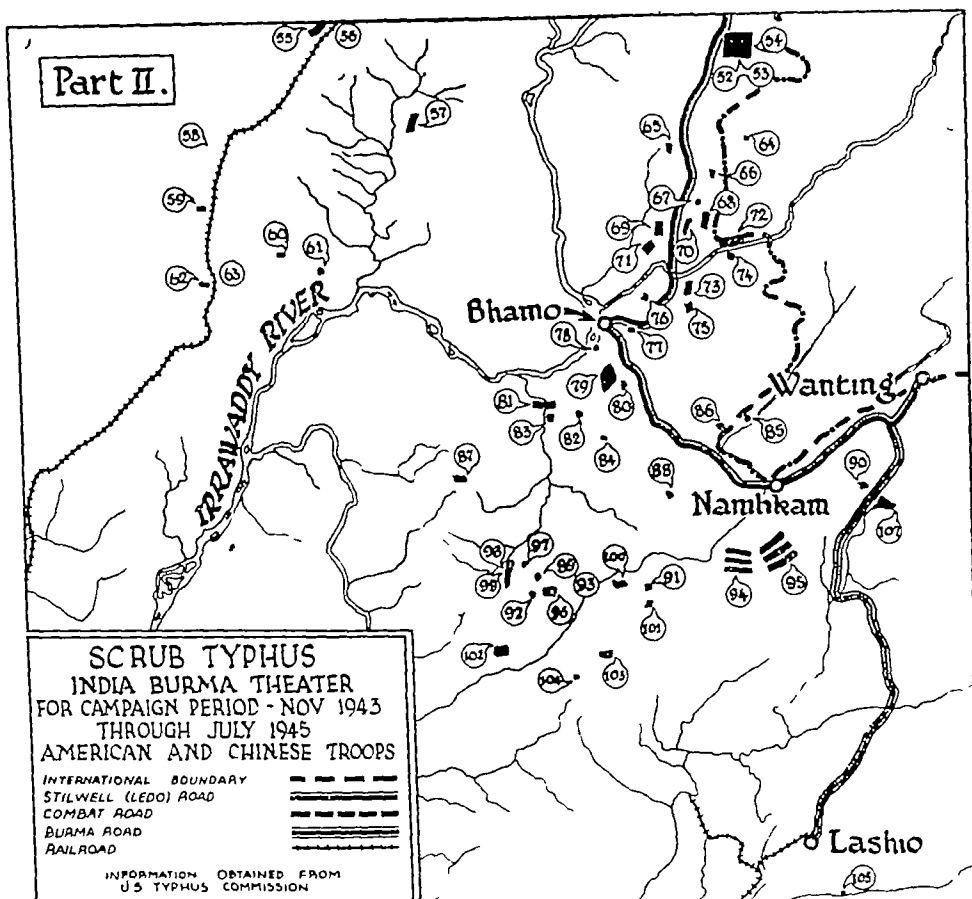
#### KEY FOR FIG. 1 (Maps, Parts I and II).

##### GEOGRAPHICAL DISTRIBUTION OF SCRUB TYPHUS IN ASIA AND BURMA.

The Map References refer to the Sectional quarter inch Map of India and Burma "Published for the Government of Burma Under the Direction of the Surveyor-General of India." Mile marks refer to the mile marks on the Stilwell Road (Ledo Road).

Area.	Map Reference.	Number of Cases.	Deaths.
1	nR 1369 Margherita, Assam	2	0
2	nR 2070 Ledo, Assam	11	1
3	nR 3074 mile 4-6	10	1

FIG 1 PART II



KEY FOR FIG 1—Continued

Area		Map Reference	Number of Cases	Deaths
4	nR	2870 Lehkapani, Assam	18	0
5	nR	3881 mile 12-15	99	12
6	nR	5278 mile 21-22	90	7
7	nS	9987 Fort Hertz area	5	1
8	nR	6174 mile 26	1	0
9	nR	7261 mile 47	1	0
10	nR	8060 mile 52	1	0
11	nR	7949 mile 58	1	0
12	nR	7528 mile 75	2	2
13	nR	8041	2	0
14	nR	8515	1	0
15	nR	7505 Shungbwyiyang area	7	0
16	nW	7598 " "	26	0
17	nW	8194	1	0
18	nX	0587 Yupbang area	2	0
19	nX	0778 mile 119, Taihpa area	4	0
20	nX	1957 Maingkwan area	3	0



KEY FOR FIG. 1—Continued

Area.	Map Reference.	Number of Cases.	Deaths.
21	nX 2243 Walebum area	1	0
22	nX 7827 Salawng Hyikang	32	8
23	nX 9222 Salawng Hyikang to Ratpong	88	18
24	nX 3528 Tingkawik Sakin area	2	0
25	nY 2232	1	0
26	nX 2906 Shadastup area	11	0
27	nX 9312 Ritpong area	1	0
28	nX 3002 Laban area	1	0
29	nX 3218	1	0
30	nX 8411 Pala area	2	0
31	nX 6814 Tategalhtawng area		0
32	nX 5308 Nhpum Ga area	6	0
33	sC 5293	15	0
34	sC 4883 Waron area	1	0
35	sC 2595 Warastup area	3	0
36	sC 3590	3	0
37	sC 2363	2	1
38	sC 4078	2	0
39	sC 3762 Komeing area	3	0
40	sC 4252	2	0
41	sC 4347	1	0
42	sC 4935 Seton area	3	0
43	sC 2423	1	1
44	sD 1862 Camp Landis		6
	sD 1453 North Airstrip, Myitkyina	233	
45	sD 0451 Myitkyina area	1	0
46	sD 1345 South Airstrip, Myitkyina	14	0
47	sD 1252 Myitkyina area	8	0
48	sD 1845 Walogmaw area	2	0
49	sD 1351 Myitkyina area	2	0
50	sD 2553	1	0
51	sD 3531	5	1
52	sD 3505	2	0
53	sD 3501	35	8
54	sD 3301	2	1
55	sH 1906 Hopin area	1	0
56	sH 4466	9	3
57	sH 2067 Hopin area	1	0
58	sG 8555 Mawhum area	1	0
59	sG 8136 Mawhu	2	0
60	sH 0730	2	0
61	sH Manhlawa area	1	0
62	sG 8614 Pwaw area	1	0
63	sG 8315	1	0
64	sJ 4070	2	0
65	sJ 2760	2	0
66	sJ 2690	1	0
67	sJ 2163	11	2
68	sJ 3448	11	0
69	sJ 1941	1	0
70	sJ 2141 Ta'h area	6	0
71	sJ 2545	6	0
72	sJ 1638	3	0
73	sJ 2133 Bhawo area	1	0
74	sJ 3635	2	0
75	sJ 2628	1	1
76	sJ 1834		

## KEY FOR FIG 1—Continued

Area		Map Reference	Number of Cases	Deaths
77	sJ	1511 Momauk area	1	1
78	sJ	0505	1	0
79	sO	0795	23	1
80	sO	1094	2	0
81	sN	8185	8	1
82	sN	9485	1	0
83	sN	8585	1	0
84	sO	0476	1	0
85	sO	4275	1	0
86	sO	3971	1	0
87	sN	5861	2	0
88	sO	1859	1	0
89	sN	8448	1	0
90	sO	7045 Hosi area	1	0
91	sO	1845	1	0
92	sN	8737	1	0
93	sN	9237	3	0
94	sO	4837 Mongwi area	17	3
95	sO	3735	51	5
96	sN	8135	1	0
97	sN	8933 Tongkwa area	11	0
98	sN	7832 Mo-Hlaing area	1	0
99	sN	7730 Tonakwa	15	0
100	sO	0628 Yepang, near Namchit River	5	0
101	sO	1126	1	0
102	sN	7712 Mannwan area	5	0
103	sO	0410 Manna area	2	0
104	sN	9804	1	0
105	sT	8334	1	0
106	tA	0929 Paoshan (not shown on map)	1	1
107	sO	6744 Namkham area	11	2
Total			1,041	91

## SEASONAL INCIDENCE

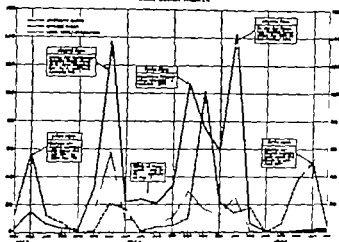
When the cases are arranged by months in accordance with the dates of admission to the hospital five incidence peaks appear (Fig 2 and Table I). These are distributed as follows: December, 1943, May, 1944, October, 1944, January, 1945, and June, 1945, suggesting a possible seasonal distribution with peaks occurring in the spring at the onset of the monsoon and again in the autumn after the end of the rains. Analysis of the military activities of the units from which the cases came, however, calls such an interpretation seriously into question.

The first peak, in November and December, 1943, principally affected the Chinese 22nd Division which was camped along the Namchik River between mile 20 and mile 24 of the Stilwell Road east of Ledo, where the division was engaged in combat and jungle training in the immediately surrounding country. This unit provided sixty-six of the eighty-five Chinese cases included in this outbreak.

The second peak, in May of 1944, coincides with the operation against Myittha by the Galahad Force and affected primarily American personnel

All but eleven of the 187 American cases were members of this combat unit. The period involved covers the time from the departure from Walahum, 4th March, 1944, to the capture of the South Alstrip at Myitkyina on the 17th May 1944. The majority of the cases apparently originated in the Selawng Hyikang and Rutpong areas between Warazup and the Irrawaddy Valley. The precipitate drop in the incidence of the disease in June coincided with a marked change in the character of the military operations. Although the South Alstrip was taken, the Japanese remained in possession of the town. Combat activities became static and were restricted to infantry patrol actions and extensive air bombing of the town and the surrounding Japanese positions.

FIG. 2.

SCRUB TYPHUS  
IN THE BURMA THEATRE

Scrub typhus in India-Burma theatre from November 1943 to June, 1945. Graphs of case incidence in American and Chinese troops, and rate per 1 000 per annum in American troops. The five peaks of incidence occurred in association with the military operational activities indicated on the chart.

Following the capture of the North Alstrip on 3rd August, the Japanese withdrew from the town and the first phase of the campaign in Upper Burma was terminated.

The third peak likewise affected American troops from mid-September to mid-November 1944. It involved the 5332 Provisional Brigade (Mara) comprising approximately 6 000 U.S. troops and 2,000 Chinese (1st Independent Regiment). Of 219 cases occurring among the American forces in September, October and November 201 were members of this unit. These troops were camped between the Samprabum Road and the Irrawaddy River approximately 8 miles to the north of the town of Myitkyina and were under going combat training and manoeuvres in the surrounding country. These activities were conducted over a relatively wide area, simulating combat conditions. The termination of this outbreak coincides with completion of the

training programme and the movement of troops to the south in preparation for the subsequent attack on Bhamo, which began in early December coincident with the initial rise of the fourth peak of the disease.

The distribution of cases of scrub typhus comprising this fourth peak resembles the distribution pattern in the Myitkyina campaign, 185 of the 194

TABLE I  
SCRUB TYPHUS IN INDIA-BURMA THEATRE  
American and Chinese case incidence and American rate (see Fig. 2) and case fatality rates

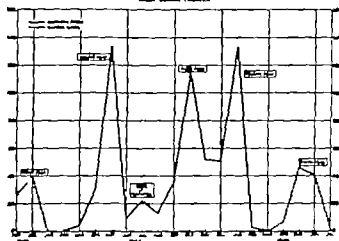
Date	Cases		Deaths		Case rate per 1,000 per annum American	Strength American Ledo area and Burma
	American	Chinese	American	Chinese		
1943—Nov	2	14	0	0		
Dec	15	57	1	1		
1944—Jan	6	12	0	0	2.73	25,990
Feb	3	7	0	1	1.29	27,101
Mar	2	0	0	0	0.88	27,000
Apr	32	1	2	0	13.82	27,919
May	137	21	25	3	57.54	28,454
June	22	17	0	2	8.97	29,351
July	24	1	0	0	8.64	32,823
Aug	21	4	1	1	7.05	35,029
Sept	34	5	1	1	10.20	39,817
Oct	107	10	3	3	20.53	43,429
Nov	75	102	2	12	17.10	50,513
Dec	59	22	2	3	12.03	57,118
1945—Jan	143	14	17	2	25.74	65,243
Feb	6	18	0	0	1.00	69,571
Mar	1	0	0	0	0.16	71,522
April	0	6	0	2	0	70,912
May	1	37	0	3	0.16	67,394
June	2	51	2	4	0.40	56,103
July	2	4	2	2	0.43	54,949
Totals	604	403	58	40	—	—
Case fatality rate	—	—	8.3%	9.9%	—	—

American cases occurring in December, 1944, and January and February, 1945, came from combat units in action against the Japanese in the Central Burma campaign. The remaining nine cases came from non-combat units which were scattered along the course of the Stilwell Road from Ledo to the vicinity of Bhamo.

The fifth peak was restricted entirely to Chinese troops again camped along the Stilwell Road in the Tirap and Nanchik valleys where they were occupying many of the camp sites from which the cases came in the initial outbreak in November and December 1943. These units were in process of staging for the return to China and were not engaged in combat training.<sup>6</sup> This outbreak of the disease will be discussed in detail subsequently.

The case incidence among American troops bore no relation to the total military population in the Advance Section, the Ledo areas of Assam and Burma. Thus the peak of 137 cases in May 1944 occurred with a military population of 23,000 while the peak of January 1945, yielded only 143 cases from a population of 65,000 troops. However when the incidence among men engaged in combat or combat training is contrasted with the incidence among non-combat personnel, striking and significant differences are revealed. The data concerning the activities of 699 of the American cases were sufficiently complete and accurate to permit this type of analysis. (Fig 3 and Table II.)

FIG. 3.  
SCRUB TYPHUS  
INDIA BURMA THEATRE



Scrub typhus in the India Burma theatre from November 1943 to June, 1945. Graphs of actual case incidence in American and Chinese troops that were directly connected with the operational activities responsible for the five peaks shown in Fig. 2.

When the "combat" cases are plotted by months, the resulting curve is almost identical with the incidence curve in Fig 2, the peaks in each corresponding to the active phases of the military operations. This relationship is more strikingly emphasized by the distribution of cases in the outbreak in January 1945. At that time there were 65,000 U.S. troops in Assam and Burma of which only 6,000 were in combat. Yet 185 of the 194 cases comprising this peak came from this combat force.

When the cases from the non-combat troops are similarly plotted by

TABLE II

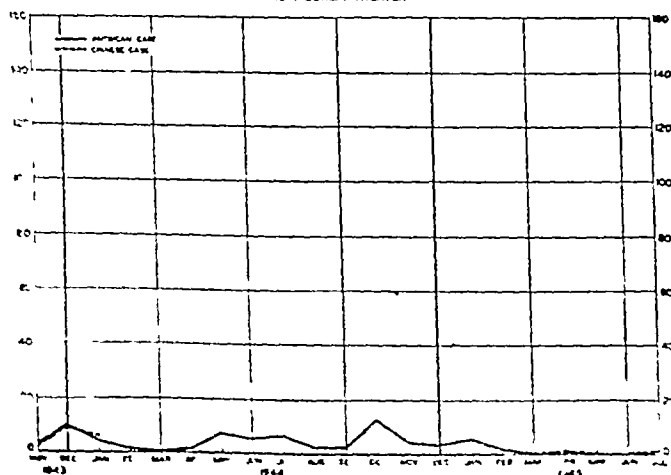
ACTUAL CASE INCIDENCE OF SCRUB TYPHUS IN TROOPS IN INDIA-BURMA THEATRE DIRECTLY CONNECTED WITH THE OPERATIONAL ACTIVITIES RESPONSIBLE FOR THE FIVE PEAKS (SEE FIGS 2 AND 3)

Date	Cases	
	American	Chinese
1943—Nov		26
Dec		40
1944—Jan		0
Feb		
Mar	4	
April	30	
May	133	
June	9	
July	22	
Aug	13	
Sept	35	
Oct	114	

Date	Cases	
	American	Chinese
1944—Nov	52	
Dec	50	
1945—Jan	133	
Feb	2	
Mar		0
April		6
May		46
June		41
July		3
Totals	597	162

months, a flat curve without significant peaks is obtained with the cases distributed throughout the year (Fig 4 and Table III) It is likewise significant that this group which comprised the great majority of the troops in the Advance Section yielded the smallest number of cases The personnel in the group includes service troops, engineers engaged in road maintenance, the pipe line units, and signal corps troops operating and maintaining the telephone lines

FIG 4  
SCRUB TYPHUS  
INDIA-BURMA THEATRE



Scrub typhus in India-Burma theatre from November, 1943, to June, 1945. Actual case incidence in American and Chinese troops that were not connected with the operational activities indicated in Fig 2

TABLE III.

ACTUAL CASE INCIDENCE OF SCRUB TYPHUS IN AMERICAN AND CHINESE TROOPS IN INDIA-BURMA THEATRE THA WERE NOT DIRECTLY CONNECTED WITH OPERATIONAL ACTIVITIES RESPONSIBLE FOR THE FIVE PEAKS. (SEE FIGS. 2, 3 AND 4)

Date.	Cases.		Date.	Cases.	
	American.	Chinese.		American.	Chinese.
1943—Nov	3	3	1944—Nov	4	
Dec.	10	9	Dec.	3	
1944—Jan.	4	6	1945—Jan.	5	
Feb.	1		Feb.	1	
Mar	0		Mar	0	0
April	1		April	0	1
May	7		May	0	0
June	8		June	0	0
July	6		July	0	1
Aug	2				
Sept.	2				
Oct.	12		Totals	66	19

These data lead inescapably to the conclusion that in the regions under consideration, Assam and Upper Burma, scrub typhus may be acquired throughout the year and that there was no true seasonal incidence. It appears that the determining factor was the character of the military operations. As would be anticipated, the greatest risk is presented by combat and the training conditions simulating combat in infective terrain.

#### THE LEDO OUTBREAK.

Cases of scrub typhus began to appear shortly after the arrival of United States and Chinese troops in the vicinity of Ledo Assam, the main staging area and advanced supply base of the United States Forces operating in the China-Burma India theatre. The areas immediately to the east along the first 25 miles of the Stilwell Road were the staging and one of the main training areas for Chinese troops flown over the "Hump" from China to participate in the campaigns of 1944 and 1945. The medical data concerning the various units occupying these camps and depots are a matter of record and it was possible with the assistance of the Headquarters N.C.A.C. (Northern Combat Area Command), Headquarters Chinese Army in India, from the records of the Surgeon, and from data taken from individual case records in the various hospitals to reconstruct the medical history of the local areas with reasonable accuracy. It is important, however to emphasize again that the figures presented are undoubtedly low because of the destruction of the hospital charts of some

three hundred Chinese patients hospitalized in the early stages of the military operations

The town of Ledo lies on the edge of the Brahmaputra River Valley at the foot of the Patkai range of mountains, in close proximity to the Tirap River which winds through the cultivated flat land. It is a tea and a rice growing area, closely approximated to the forest-covered foothills. Sixteen cases of the disease were acquired in this immediate region from November of 1943 through June of 1945 (Table IV). Two of these cases occurred among Chinese anti-aircraft gunners stationed about the periphery of the Ledo Airstrip adjacent to the town. Three cases came from the personnel of another anti-aircraft battalion which had its gun emplacements scattered from Margherita to Lehkapani east of Ledo, and two from a signal battalion stationed at Lehkapani. The 4.6 mile mark camp situated just beyond the main warehouse area of the

TABLE IV  
INCIDENCE OF SCRUB TYPHUS IN THE VICINITY OF LEDO, ASSAM

Site	Periods occupied	Occurrence of cases	Number of cases
Ledo Airstrip	1943 onwards	June, December, 1944	2
Lehkapani	1943 onwards	November, December, 1943	3
		January, June, 1944	2
		January, 1945	1
4.6 mile mark	October, 1943, onwards	October, 1944	1
6.0 mile mark	October, 1943, onwards	November, December, 1943	2
		January, February, 1944	5

Lehkapani supply base was occupied by a Chinese motor transport regiment continuously from October, 1943, to the conclusion of these studies. One case occurred in this unit. Seven cases came from the 6.0 mile mark installation which was occupied from the autumn of 1943 onwards by the Headquarters, Chinese Army in India. It is of interest that all occurred in the early months of occupancy, the period of clearing the land and of construction of the buildings.

The relatively small number of cases originating in this Ledo-Lehkapani area cannot be accepted as a measure of the endemicity of the disease. Although the military population was large and the case incidence low, the majority of the troops stationed here were on duty at the headquarters or in the supply depots, and not engaged in training activities. The distribution of the cases



in point of time and the duties of the individual soldiers again indicate that the type of duty was an important factor determining the risk of infection.

A sharp outbreak of scrub typhus among Chinese troops camped along the Stilwell Road between the 8.5 and the 23 mile marks in the spring of 1945 provided exceptional opportunities for investigation of local endemicity vectors and possible reservoirs. The units concerned had recently arrived from Ramgarh, India, and from Burma for staging preparatory to their return to China. They were not engaged in training activities and consequently it appeared that the infection must have been acquired in close proximity to the individual camp sites. The epidemic began in April and terminated abruptly in the latter part of June. In all, there were ninety five cases with eleven deaths. Throughout the total period of occupancy from January 1943 to the summer of 1945 190 verified cases were attributable to these areas. (Table V)

TABLE V

INCIDENCE OF SCRUB TYPHUS IN CHINESE ARMY CAMP SITES ALONG THE STILLWELL ROAD.

Sta.	Period occupied.	Occurrence of cases.	No. of cases.
8.5 mile mark	July to November 1943	—	0
	February to June, 1945	May	1
11.8 to 11.9 mile mark	November 1943, to ?	—	0
	April, 1944, to June, 1945	May 1945	8
12.0 mile mark	July to December 1943	October November	3
	February to December 1944	—	0
	January to June, 1945	April, May June	26
12.4 mile mark	July 1943, to September 1944	—	0
	March to June 1945	April, May June	34
13.6 mile mark	January to September 1944	—	8
	November 1944, to June 1945	November December	2
13.8 mile mark	July 1943, to June 1945	January 1944	1
		June, 1945	1
14.8 mile mark	May to June, 1945	May June	5
21 to 23 mile mark	October 1943, to January 1944	November December	66
		September October	4
	April to May 1945	November December April, May	20

The terrain in which these camps were located falls into three natural divisions. The first, extending to the 15 mile mark, is flat country through which the road for the most part closely parallels the Tirap River and the camp sites were placed between the road and the river bank. It is densely covered with bamboo, tall trees and intermediate growth. Air photographs show only a few small isolated areas of what appear to be old cultivated land covered with second growth. The intermediate section lying between the 15 and the 19 mile marks is composed of low rolling forest-covered hills. The cover is primary regrowth jungle mingled with patches of bamboo. There is no old or recently cultivated land. Only one unit was situated in this section, a U S engineer battalion, which will be referred to subsequently. At approximately the 19 mile mark the road leaves the low hills to enter the valley of the Namchik River. From this point it traverses gently rolling country in close proximity to the river. At approximately the 22½ mile mark it crosses the river and winds gradually upward into the foothills of the Patkai Mountains. The flora in this region does not differ from that already described with the exception that there is more evidence of old cultivation on the air photographs. Such areas, however, are neither numerous nor large. The individual camp sites so far as could be ascertained from information obtained at the various headquarters and from study of air photographs taken in November, 1943, were cut from the forest and not placed on previously cleared land.

Certain features of the individual camps are of interest and possible importance in connection with the epidemiology. The 8.5 mile mark camp was situated well back from the road, between it and the Tirap River. There was much high grass about the tentage area and on the sloping river bank. Although this was occupied from July to November of 1943, no cases are recorded from it at that time and only one case occurred in the spring of 1945.

The installation at the 11.8 to 11.9 mile mark appeared to be cleared from unbroken jungle comprised largely of bamboo with scattered large trees. It was occupied in November, 1943, and continuously from April, 1944, to June, 1945. The river bank in this area is high and abrupt. Although no cases were recorded prior to 1945, there were eight in May of that year.

The 12.0 mile mark camp presents no significant differences. It was occupied almost continuously from July, 1943. Two cases of scrub typhus are reported from it in October and November of 1943, none, however, during 1944. In the outbreak in the spring of 1945 it appeared as one of the most heavily infected foci with twenty-six cases in April, May and June.

The immediately adjacent 12.4 mile mark camp, although occupied in 1943, yielded no recorded cases at that time. In the spring of 1945, however, thirty-four cases occurred in the unit located on this site. It differs in one respect from the adjacent one. A path leading from the tents gave access to an area at the river's edge, covered with grass and brush, which appeared to be a popular bathing site at the time of the 1945 epidemic. It was also used

by the Chinese soldiers for washing uniforms which were customarily spread on the grass or brush to dry

The 13.6 mile mark camp presents no noticeably different features as regards the general character of the terrain, apart from scrub growth in previously occupied areas contiguous to the tentage areas in the spring of 1945 and a very restricted cleared area of river bank. During occupancy from January to September 1944 no cases were recorded. In November and December of that year there were two cases but none developed during June, the only month in which it was used in 1945. The immediately adjacent 13.6 mile camp, although occupied continuously from July of 1943 through June of 1945 yielded only two cases which occurred respectively in January of 1944 and June of 1945.

It was not possible to obtain the history of the camp site at the 14.5 mile mark prior to 1945 although it was evident from inspection of the ground that it had been extensively used in the past. At the time of the 1945 outbreak, the tent area extended along the edge of the river for some 200 yards as well as along the access way to the Stilwell Road. It had been cleared from primary forest of large timber. Five cases of the disease occurred at this site in May and June of 1945.

The area from the 21 to the 23 mile marks in the Namchik River valley was a heavily infected region. Although the majority of the camp sites were cut from virgin jungle the early air photographs show evidence of previous clearing of some small areas which had subsequently been covered with second growth scrub. The 22nd Chinese Division moved into this area in October of 1943 for jungle training prior to entering combat in Burma. A sharp outbreak of the disease, characterized by a rapid rise and an equally rapid decline, occurred between 20th November 1943 and 8th January 1944. There was a total of sixty-six cases in this period. Since the records indicate that the division was moved into the forward combat areas in "early January" it is possible that this movement accounted for the abrupt termination of the outbreak.

The 14th Chinese Division entered this same area in April of 1944. Two of the three regiments moved forward to combat in July while Division Headquarters, one regiment and some miscellaneous troops, remained in the area until the last week of November when they in turn began to move into Burma. While in the Namchik region the division was undergoing combat and jungle training. Twenty-four cases of scrub typhus occurred during the autumn months.

The twenty cases in April and May of 1945 were among the personnel of a unit which established its camp at the 21 mile mark on the site of a ration dump which was abandoned in December of 1944. Large quantities of spoiled grains and other stores were left uncovered until early in 1945 when the area was bulldozed, part of the refuse covered with earth and part pushed over

the bank of the river where some remained exposed. The entire area was heavily infested with rats and when the first cases of the disease appeared the site was abandoned.

Three United States Army Units located in the central hilly area between the Tirap and the Namchik valleys provided an interesting and important set of controls. A quartermaster Remount Depot at the 15.0 mile mark was established in the autumn of 1943 and remained continuously occupied. It was immediately on the bank of the Tirap River and the terrain did not differ from that of the nearby Chinese Army camps. At the 15.5 mile mark there was a battalion of road engineers and an American evacuation hospital was established in the autumn of 1943 at the 19 mile mark close to the Namchik River. There were no cases of the disease among the personnel of these units until the late summer of 1945 when one case occurred among the hospital complement in an individual who had been fishing along the river.

Comparison of the activities of the Chinese and American personnel and of the respective camps provides interesting contrasts and similarities. During the period of the outbreak in the spring of 1945 the military duties of the men in the different units did not vary sufficiently to be the cause of probable differences in degree of exposure to infection. No training activities requiring work in the forest were in force, and there was little temptation to the U.S. soldiers to leave the roads and beaten paths. Swimming in the river was not common practice since all the U.S. installations were equipped with running water and adequate bathing facilities. The Chinese, on the other hand, were accustomed to forage constantly in the jungle for edible plants to supplement their rations. They bathed regularly in the adjacent rivers and were accustomed to dry their uniforms and other garments on the grass and bushes while bathing. These facts, together with the almost universal practice of wearing shorts, provided frequent and maximal exposure to trombiculid mites.

A further difference lay in the camp sanitation. While the actual tentage area of the Chinese units was immaculate, the surrounding brush and jungle was a mass of rubbish and food remnants. Rats were present in large numbers in most of the camps but were also present in considerable numbers in the American camps where the environmental sanitation and rubbish disposal were much better carried out. Differences in the rat and mite populations did not seem sufficient to explain the strikingly different incidence of the disease.

Neither the rodent population, the sanitation, nor the foraging in the jungle is sufficient to account for the markedly greater number of cases in 1945 coming from the 12.0 and the 12.4 mile mark camps in contrast to those from the other camp sites. These two adjacent areas differed from the others in one important respect, the presence of the small grassy area at the river's edge which was used for bathing and the washing of clothing. It is probable that soldiers from both camps used this bathing area in preference to others. This area on investigation was found to be heavily infested with Trombiculidae.

Generally speaking the river banks at the other camps were steep and without grass-covered flats adjacent to the water edge.

The high case incidence in 1943 and 1944 at the 20 to 23 mile marks in the Namchik Valley must be attributed to operational activities, since the troops stationed there at that time were undergoing intensive training in preparation for combat. These figures, therefore while of interest in connection with the endemicity are not relevant to the incidence figures in the spring of 1945. They provide important evidence, however of the period through which the infection may persist and remain a hazard to man, and support the thesis that once the disease makes its appearance the terrain concerned should be held suspect for at least a year.

As was the case in 1943 the termination of the 1945 outbreak coincided with the rapid dispatch of the units to China. This evacuation of the endemic areas is almost certainly the explanation for the rapid subsidence of the disease since recommended precautionary measures were not carried out effectively.

#### VECTORS AND RESERVOIRS.

Investigations into the epidemiology of scrub typhus in Assam and Burma were handicapped at the start by lack of information concerning the species of trombiculid mites indigenous to the region. This entailed an extensive taxonomic study before evaluation of possible vectors of the disease could be undertaken. These studies were conducted continuously from November 1944, to November 1945. Thirteen genera of trombiculid mites were identified, four of which are new and undescribed. Preliminary studies indicate that forty five new species are included within these thirteen genera. It is of interest that scrub itch is not known in this region. Boot collection proved to be an ineffective technique for obtaining samples of mites and for the most part it was necessary to recover them from their natural hosts.

Previous workers on an epidemiological basis had incriminated *T. deliensis* Welch (syn. *T. walchi* Womersley and Henslip 1943) as the vector of scrub typhus in Sumatra and Malaya. In the present studies this mite was found to be the commonest species during the months from May to November in areas in Assam and Burma where scrub typhus cases were known to have originated, and though difficult to find in certain areas, it was found in others throughout the year. Therefore, it seemed imperative to attempt isolation of strains of *R. orientalis* from this species in Assam and Burma.

Because of the high degree of susceptibility of the gerbille to infection by *R. orientalis* it was decided to use this animal for primary isolation. Adequate numbers of *Gerbillus gerbillus* and *G. pyramiden* were made available through the co-operation of the Field Headquarters of the U.S. of America Typhus Commission in Cairo. The following methods were used for the recovery of the rickettsiae from mites and mammals. Paired experiments were conducted in many instances. Mites taken from trapped rats or shrews, after

of the exudate to two gerbilles confirmed the observation. This experiment, therefore, demonstrates that transovarial transmission of *Rickettsia orientalis* occurs in *Trombicula deliensis*, and that this species of mite transmits the infection during feeding.

Further evidence was obtained in the course of experiments designed to investigate the possibility that incompletely engorged mites may, under certain conditions, detach from the initial host and re-attach to another. The ear of a trapped wild rodent with many mites attached was removed and placed in a vial for 24 hours. At the end of this period, many partially engorged larvae were found free in the vial. When removed and inserted into the ear of a white rat some promptly re-attached. In the course of four such experiments conducted with larvae of *T. deliensis*, the animal subsequently became infected and the characteristic rickettsiae were demonstrated.

Much more difficulty was encountered in the isolation of the rickettsiae from mammalian hosts and the number of recoveries was much smaller. Only two species of mammals were found to be infected, the Yunnan buff-breasted rat, *Rattus flavpectus yunnanensis* (Anderson) in the Ledo Assam area, and the Assamese tree shrew, *Tupaia belangeri versurae* Thomas in the Myitkyina region. *R. yunnanensis* was the principal species of wild rat found in Assam and was frequently trapped in Burma. In the latter region *Rattus concolor concolor* Blyth, the little Burmese rat, was found to be abundant in and around the native villages. However, it was rarely infested with mites and rickettsiae could not be recovered from it. Consequently, it is of doubtful importance in the epidemiology of the disease. *Rattus rattus sladem* (Anderson), Sladen's roof rat, was likewise found to be common and widely distributed in the Myitkyina region, both in the field and about buildings. It was frequently infested with *T. deliensis* and three strains of *R. orientalis* were recovered from mites removed from this host. Although rickettsiae were not isolated from *R. r. sladem*, the number of animals examined was too small to be significant.

The exposure of experimental animals in nature, both the common laboratory white rat and the gerbille, was found to be a useful procedure in the exact localization of foci of infected mites. Three strains of *R. orientalis* were obtained by this technique in the investigations of the camps along the Stilwell Road, and one strain in the Myitkyina region. A pen measuring approximately 10 x 10 feet square was made by inserting iron sheets on edge into the top soil and the animals were released in the enclosure where they were permitted to remain for periods of several hours. Strains were recovered from three of the white rats, from the pooled tissues of four gerbilles, and from a lot of mites collected from a fourth white rat.

#### NATURAL HOSTS OF *Trombicula deliensis* IN ASSAM AND BURMA

*T. deliensis* was found to attach in nature to a variety of avian and mammalian hosts in addition to man (Table VIII). It was not practicable to



indicated by rickettsial isolation experiments, and the rodent infection rate similarly determined. Evaluation of these data is arbitrarily expressed as the "Established Endemicity" (Table IX.)

The evidence already presented renders it inadvisable to consider the infection to be absent from any of the areas studied, therefore the camp at

TABLE IX.  
'ESTABLISHED ENDEMICITY' OF SCRUB TYPHUS ALONG THE STILWELL ROAD

Area	Organization	Attack rate *	Mite strains			Rodent strains			"Established Endemicity"
			No at-tempts	No positive	% positive	No at-tempts	No positive	% positive	
15.5 mile mark	352 Eng Bn	0	20	0	0	20	0	0	Endemic ?
15.0 mile mark	698 QM Re-mount	0	18	1	6	20	0	0	Endemic
4.6 mile mark	6th Motor Regiment, Chinese	0	12	2	15	12	0	0	Endemic
22 mile mark	5th FA, Chinese	46	11	3	27	11	0	0	Hyper-endemic ?
16 mile mark	Garbage dump	No troops	13	2	15	18	1	5 +	Hyper-endemic ?
19 mile mark	14th Evac Hospital	0	4	1	25	11	1	9	Hyper-endemic ?
12 mile mark	12th FA	98	6	4	66	10	0	0	Hyper-endemic
	1st Tank Bn, Chinese	574							
12.4 mile mark	3rd Tank Bn, Chinese	600	29	10	34	23	1	4	Hyper-endemic
21 mile mark	1st Motor Regiment, Chinese	1,040, then abandoned	40	17	42	41	5	12.5	Hyper-endemic

$$\text{* Four-weekly attack rate per 1,000 exposed per annum} = \frac{1,000 \times \text{cases} \times 52 \times 4}{\text{strength} \times \text{weeks exposure}}$$

the 15.5 mile mark is listed as questionably endemic despite the absence of clinical cases or mite or rodent strains of rickettsiae. The 4.6 mile mark is considered to be an endemic area in view of the recovery of the infectious agent from 15 per cent of the mite lots tested although it was not recovered from rats and no human cases were ascribed to it. Similarly the high mite



and rodent rates at the 19 mile mark over weight the low human incidence, one case, and strongly suggest hyperendemicity. In view of the 4-weekly attack rate of 600 and 1 040 per thousand per annum, the mite infection percentages of 10 per cent. and 17 per cent., and the rodent infection percentages of 4 per cent. and 12.5 per cent. respectively it seems reasonable to classify the 12.4 and the 21 mile marks as hyperendemic areas.

In Table X the percentage of *T. deliensis* found in the mite collections is contrasted with the arbitrarily expressed "Established Endemicity". It is seen that there is a rough parallelism between the prevalence of this mite and the presence of proved infection. In the three areas which gave rise to the largest numbers of human cases and which are listed as hyperendemic, *T. deliensis* constituted 60 per cent., 74 per cent., and 98 per cent. respectively of all the

TABLE X.

"ESTABLISHED ENDEMICITY" AND OCCURRENCE OF *Trituoccamushi deliensis* ALONG THE STEWELL ROAD.

Area.	Attack rate.	Mite lots positive %	Rodent lots positive %	No. of rats examined.	No. of mites examined.	% <i>Trituoccamushi deliensis</i>	"Established endemicity"
18.5 mile mark	0	0	0	31	260	42	Endemic ?
18.0 mile mark	0	6	0	41	243	13	Endemic
4.8 mile mark	0	18	0	25	426	43	Endemic
22 m l mark	48	27	0	31	213	62	Hyperendemic ?
16 mil mark	No troops in area.	18	8	41	222	89	Hyperendemic ?
19 mil mark	0	25	9	29	161	42	Hyperendemic ?
13 m l mark	98 574	66	0	20	317	60	Hyperendemic
12.4 mile mark	600	34	4	37	800	74	Hyperendemic
21 mil mark	1,040 then ban done	42	12.5	78	1,304	98	Hyperendemic

$$\text{Four week attack rate per 1,000 exposed per annum} = \frac{1,000 \times \text{cases} \times 52 \times 4}{\text{strength} \quad \text{weeks exposure}}$$

mites sampled. It is of course recognized that interpretations based only on the mite population are open to serious objection as a valid index of the presence or absence of the infection. However, in the present state of information concerning the epidemiology of scrub typhus and its wide distribution in Assam and Burma, a high incidence of *T. deliensis* in a given area renders such an area highly suspect as a potential source of human disease (Table X).

### SUMMARY

It is difficult properly to evaluate the problem of seasonal incidence. The peaks of the disease, with the exception of the one in January, 1945, roughly correspond to the beginning and the end of the monsoon. These periods are also those in which *T. deliensis* was found in greatest abundance. The disease, however, was acquired in every month of the year. These facts, together with the strict relationship between incidence rates and the type of military duty, strongly suggest that several factors participate in determining the epidemiology and that exposure of the individual to mite infestation, together with fluctuation of the *T. deliensis* population, are obviously dominant factors.

The history of the disease in the vicinity of Ledo, Assam, and in the camps along the Stilwell Road emphasizes the sharp geographical localization of the infection. It again suggests that individual activity plays a determining role and that exposure to and risk of infection vary directly with exposure to the vector mites. The risk of infection, however, can be minimized if not eliminated by proper use of simple precautionary measures.

The abrupt termination of the outbreaks in 1943 and 1945 in this region coincided with the movement of the affected troops out of the areas concerned. The persistence of the infection about individual camp sites indicates that an area in which the disease has occurred remains hazardous for at least a year.

No evidence was obtained to indicate that the risk of infection is consistently associated with a particular type of terrain.

*R. orientalis* was recovered from fifty-three lots of trombiculid mites by animal inoculation. The larval mites were obtained from trapped rats or shrews taken in their natural habitat. With one exception, *T. deliensis* constituted from 36 per cent to 100 per cent of the samples reserved from each lot for identification and in no instance were the rickettsiae isolated from lots of mites in which *T. deliensis* was absent. This species of mite was shown to attach to man under field conditions. When laboratory reared larvae, obtained in culture from adult female *T. deliensis* taken in the field, were allowed to attach to and feed upon a laboratory animal, the animal died on the 20th day and *R. orientalis* was recovered from the peritoneal and pleural exudates. This experiment demonstrates conclusively that transovarial transmission occurs in this mite. These facts, therefore, demonstrate that *T. deliensis* is the important vector of the disease in the Ledo area of Assam and Upper Burma.

The rough parallelism found between the arbitrarily expressed "Established Endemicity" based upon human case incidence and the recovery of mite and rodent strains of *R. orientalis* on the one hand, and the prevalence of *T. deliensis* on the other suggest that the relative abundance of this mite in any area may provide an approximate index of the hazard of infection.

It is generally stated that rodents constitute the reservoir of infection however since transovarial transmission has been demonstrated in *T. deliensis*, and since a rough parallelism has been shown to exist between the density of this mite and the prevalence of the disease, it seems logical to regard the mite both as an important reservoir and the vector.

The numerous mammals and birds which *T. deliensis* accepts as its natural hosts provide an obvious mechanism for the dissemination of the infected mites.

Although it has not been possible to complete the studies of the large numbers of mites collected and while it may be necessary subsequently to modify certain of the data presented in this communication especially with reference to species percentage the following statements and conclusions seem warranted —

#### CONCLUSIONS

1 Tautsugamushi disease (scrub typhus) is widely distributed in the Ledo area of Assam and in Upper Burma.

2 *Trombicula deliensis* Walch was shown to attach to man under natural field conditions.

3 It was shown that *Trombicula deliensis* Walch transmits *Rickettsia orientalis* to susceptible experimental animals during feeding.

4 *Trombicula deliensis* Walch is an important vector of the disease in these areas. No evidence was obtained to incriminate any other species of mite.

5 Experimental proof was obtained of transovarial transmission of *Rickettsia orientalis* in *Trombicula deliensis* Walch.

6 *Rattus flavespectus yunnanensis* (Anderson) the Yunnan buff breasted rat, and *Tapia belangeri cerverae* Thomas, the Assamese tree shrew were the only mammals found to be naturally infected with *Rickettsia orientalis*.

7 There appeared to be no strict seasonal distribution of the disease in man and it may be acquired throughout the year.

8. The density of the *Trombicula deliensis* Walch population in any area may provide an approximate index of the risk of infection.

9 Terrain in which cases of the disease have occurred remains hazardous for at least 1 year.

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## DISCUSSION

Dr Kenneth Mellanby. It is a great privilege for me to open the discussion this evening on Colonel MACKIE's exceedingly interesting paper. I owe so much to the kindness and co-operation of the United States Typhus Commission. I have enjoyed their hospitality in New Guinea, in America, and particularly from Colonel MACKIE's own unit in Burma. Colonel MACKIE has clearly shown what a fine and substantial contribution his commission has made to our knowledge of scrub typhus. Previously we depended mainly on theories and suppositions. We had the results of the original Japanese investigations, and the excellent work of my colleague, Dr LEWTHWAITE (who we all regret is not here this evening) and his co-workers in Malaya, but we mainly depended on circumstantial evidence. Colonel MACKIE has shown us that transovarial transmission for instance has been definitely established, and that the mite vector has been clearly incriminated. It may be that further work will make it necessary to modify our views on the systematics of the trombiculid

mites which carry the disease. In any such cases will only be in matters of detail and will not show how it was that the (Colonel Mac) it has put forward.

My experience of trying to work with the mites, how, how careful one must be in trying to apply the results of laboratory experiments to practical problems, is the result. There has been some controversy as to whether mites which have once fed on a host will ever reattach to another. Thus if a rat infested with *Leishmania* is killed in a trap, what happens to the mites on its body? I have not taken any field observations of them to turn them into nymphs. Some workers have suggested that they all die, others that they will hold for a new host. I have not proved or refuted them in this way, but in a camp we have seen rather than decrease the danger of scrub typhus.

On the real matter of the dead bandicoots covered with hundreds of practically useful mites. I have seen places in Japan, either with live, unfed rats. So one mite is attached to the second animal. At first this seemed good evidence to show that the mites could not be a danger, but in fact it is a very bad experiment. Professor Huxford, the head of my department, has on several occasions referred to this Society the importance of studying the exact temperature and humidity to which insects and other disease carrying arthropods are subjected. In the experiment just mentioned the humidity was too great. Under other circumstances similar to those mentioned by Colonel MacCall, it proved possible to procure to reattach in considerable numbers, and there seems no doubt that badly run anti-rat measures may sometimes increase rather than decrease the danger.

Colonel MacCall has only just referred to control measures, but his commission has made considerable progress in the matter. Some excellent work on the same subject has also been done by the Australian forces, particularly by Major McCulloch. The method of protecting our men in South-East Asia from mites and scrub typhus was based on McCulloch's work, and the training film I shall now show demonstrates his method. In the film we use dibutyl phthalate, better work suggests that benzyl benzoate may be even better. Incidentally, this film was intended for a lay audience but may be of some interest tonight in explaining the technique of infection.

The film, "The Prevention of Scrub Typhus," was then shown.

*Notes*—This film was intended to explain to the Army in S.E. Asia just what scrub typhus is, how it is contracted and how infection may be avoided. It was felt that an understanding of the problem would reduce the "mystery" of the disease, and be good for morale.

Microphotographs of living *Trambicula deliensis* show how the infesting mite reaches man and spreads the disease. Dibutyl phthalate is shown to be lethal to mites, which are killed when they fall over impregnated cloth. A simple technique by which men in forward areas, with no special equipment, can treat their garments to make them mite-proof is then shown.

Two ounces of dibutyl phthalate (DBP) is sufficient to treat two sets of tropical uniform (blouse, trousers, underclothes, socks etc.). Each man is issued with the fluid which he means to use on his garments. He dips his fingers into the fluid, rubs his hands lightly together, and so obtains a thin layer over his palms and fingers. The hands are

then wiped over the cloth, which is thus smeared with the DBP. Experience has shown just how many smears are needed to cover each garment economically and efficiently—*i.e.* six smears per sock, thirty for the trousers, etc.

Finally the way in which DBP resists up to eight washes in cold water, wading through rivers, heavy rain, etc., is indicated.

The film was produced by the Directorate of Army Cinematography and shown to the Society by the kindness of the War Office and the Army Cinema Corporation. It was made at the request of the Director of Medical Services and of the Medical Advisory Division, H.Q. Supreme Allied Commander S.E. Asia, acting on the advice of the Medical Research Council Scrub Typhus Commission. The original script was written by Dr KENNETH MELLANBY who supervised the technical side of the production.

Colonel H. E. Shortt: In the first place I would like to congratulate Colonel MACKIE on the exceedingly interesting paper he has given us. It was especially interesting to myself because I have a fairly intimate knowledge of practically the whole of the ground which Colonel MACKIE dealt with. I am not talking of the ground merely in the sense of the subject matter of his discourse but actually of the ground in its literal sense, as covered on "flat feet," which is the way I did it. I first became interested in the subject of typhus many years ago. I think I saw my first case in India about 1912, and did not think much more about it until 1934-'35 and '36, when I saw a good many of the cases which have been described from the hills, the lower hills of the Himalayas. Whether the disease I saw there is exactly the same as that which is the subject of Colonel MACKIE's paper I do not know but it would appear to be very much of the same type, and probably the vector there is the same. About this time I collected all accounts in the literature which has been published of this disease in India, and at that time they amounted to something like 170 known cases of the disease. These were scattered all over India. There were one or two interesting points Colonel MACKIE has mentioned. One was that there is no seasonal variation in the disease in the region which he was dealing with but when one came to analyse the 170 cases in India which I have already alluded to one interesting fact was that the cases which occurred in the plains areas in India were nearly all those which occurred in the cold weather months, whereas the cases in the hills nearly always occurred in the hot-weather months. The reason for this I don't know but it may be again purely a coincidence because during the hot weather months the troops were in large numbers in the hills, and that the majority of cases were amongst troops thus it may not have been a seasonal variation at all, but merely a coincidence owing to the distribution of the troops at these particular times. After leaving Kasauli, the next place I went to was Madras. I knew cases had occurred in the jungle areas here, and it struck me that it would be interesting to see what the distribution was. There were only eleven or twelve cases recorded, but in the Institute where I was working we had very large numbers of blood specimens sent in for the diagnosis of the enteric group by the Widal test. Many of the results were negative, and it struck me that it would be a good idea to take the negative specimens and test them by the Weil-Felix reaction. We tested

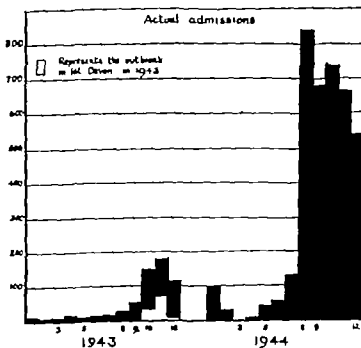
438 bloods which were negative to the ordinary Widal test for the enteric group. For the Weil-Felix reaction we took a titre of 1/200 as positive. This we decided on after testing about 200 normal cases, in none of which did we get a titre higher than 1/50, and that only in five cases. We therefore considered a titre of 1/200 a safe limit. There were sixty-five cases which gave a positive Weil-Felix reaction. Of these forty-two were of OX19 type, sixteen of OXK type, and seven of OX2 type. I would like Colonel MACKIE to tell us what the types he was dealing with were. I know the chief type was OXK, but were there any other types prevalent in the area with which he dealt? His work I know was chiefly with the United States troops, but there were a large number of other personnel working in this very area, the Ledo Road—Assamese coolies and porters from various hill tribes. They must have numbered, I think, almost as many individuals as the United States troops in this area. I was personally responsible for the health of these labour corps. There was a very large hospital under my administrative supervision at Lakhapani with an American officer in charge. I don't know whether Colonel MACKIE has any records of these people, but it would be interesting to know if they were equally affected, because they worked very closely with the troops along the same tracks, and were subject throughout the whole area he has dealt with to the same conditions as the United States troops. There was another group of people who also went along these tracks. I am talking of 1942, before there was any Ledo Road, when the whole country was traversed only by one or two tracks used by Nagas, and even these Nagas were very scanty in that country. I refer to the refugees from Burma fleeing from the Japanese under the conditions of the most awful refugees' road in the world. These refugees came along tracks which were seas of mud. Some, incredible as it may sound, were drowned in the mud, and when the labour corps I have referred to were sent along these tracks we had to put what had been primitive hill tribes into trousers to save them from the bites of insects, because when these bites were scratched they caused sores and ulcers, and into boots, because otherwise they cut their feet on the bones of the refugees in the lowest parts of the tracks. These refugees came from Myitkyina to Ledo, a distance of 130 miles, exposed to inconceivably severe conditions the whole way, and were in some cases 5 or 6 months on the road. These people, if any, should have been infected with scrub typhus. We have Dr LAPPING here with us tonight, one of those chiefly responsible for the care of these people when they reached Assam, and I will quote from a report I wrote on the exodus of refugees from Burma in which I have acknowledged his valuable work. In his report to me he says, "There have been several cases of what is best termed clinical typhus, I have not had a positive Weil-Felix, but have little doubt that they were typhus. The infection must lie outside the agglutinating range of OXK, OX19 and OX2. I would like to hear whether Dr MACKIE has any information about the possible incidence of typhus among these people who were exposed to



infection almost continuously I should think, during a period from somewhere about April or May when they left Myitkyna, until the following November or December when practically all had come through.

Lieut-Colonel M H P Sayers I would like to tell briefly how *typhus* affected us in South-East Asia, particularly with regard to its seasonal incidence. Quite early on we thought the disease as encountered in Bengal and on the Indo-Burma border showed a marked seasonal variation and subsequent experience confirmed this view. The two graphs bear what I have to say.

GRAPH 1



Scrub Typhus. Eastern Army and 14th Army

For practical purposes only the one disease was met—scrub typhus (*tsutsugamushi* fever)—with agglutination of OXK suspensions. Before recent war this disease had not been a military problem. Outbreaks began to occur among troops in Burma and Eastern India from September 1941 when over 100 cases were reported south of Mandalay. This was one of the earliest examples of a "unit outbreak" which became such a common feature of war in the Far East. There followed the retreat from Burma in the summer of 1942 and the disease was hardly heard of again until the following autumn.

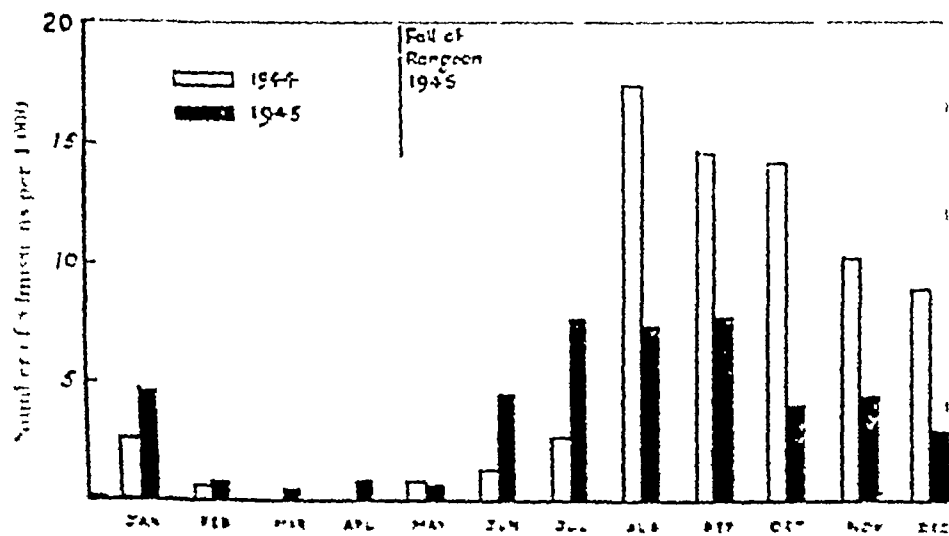
when sixteen cases occurred in a unit stationed in Calcutta. In 1943, troops began to concentrate in Eastern India for the impending operations to recover Burma.

Graph 1 shows the actual admissions by months of cases of scrub typhus in 1943 from Eastern Army and for the following year, 1944, when Eastern Army became the 14th Army. It is noted that there appears to be a very marked seasonal change. A few sporadic cases occurred during the early months of 1943, but the disease did not assume serious proportions until the autumn when a number of cases occurred, mainly in Bengal and on the Indo-Burma border in the neighbourhood of Imphal.

The peak year was 1944, when 4,000 cases occurred with some 350 deaths. Graph 1 shows a very abrupt seasonal change. The majority of these cases occurred south-east of Imphal in the Kabaw valley, that is some 300 miles south west of the area referred to by Colonel Mackie.

In 1945 we again saw an overwhelming preponderance of cases in the monsoon and post-monsoon seasons. (See Graph 2 which shows the number of admissions to hospital per 1,000 in 1944 and 1945 respectively.)

GRAPH 2.



Note: Fall of scrub typhus in all months expressed as admissions per 1,000 per month.

In any view, the American experience did not really differ from that of the British. The monsoon of 1945, Colonel Mackie had observed, had been a very bad one. In the third of the year, although the cases were not numerous, they were still present. The last year saw a very bad one, and it was not until the end of the year that the cases began to decline. In October and November, the cases were still present, but they were not as numerous as in the previous year. The cases were still present, but they were not as numerous as in the previous year.

occurred in the autumn of 1943. This concerned some eighty cases around Mandalay.

When one tries to correlate the incidence of scrub typhus in South-East Asia—I mean primarily the Indo-Burma border—with the concurrent operations, the available data are not perhaps sufficient for us to do so as accurately as Colonel MACKIE has done. But generally speaking one can say the operational season was essentially the dry weather—that is the first half of the year. That, after all, was the time when operations were more practicable. In 1943 they were mainly confined to patrol activities on the Central Front but in 1944 and 1945 some of the heaviest fighting took place during the first half of the year.

It was in March, 1944 that three divisions fought their way back to the Imphal Plain along tracks where before and since large numbers of cases of typhus have occurred, yet the incidence at this time is seen to be negligible (see Graph 2). Nor did it begin to rise until troops moved out of Imphal over these very same tracks after the siege was raised the following May. In 1945 very heavy fighting was taking place during February and March in Central and Southern Burma when our troops were advancing on Mandalay. The incidence of typhus is again seen to be low but in the latter part of the year it rose steeply.

It was difficult to avoid the conclusion that the marked change in climate which occurs in Burma was one of the factors that influenced the number of cases and we came to regard the second half of the year as the typhus season as opposed to the first half which was known as the "shooting season."

I am very sorry that Dr. LEWTHWARTZ and Lieut. Colonel AUDY of the scrub typhus research teams set up in South-East Asia Command, are not here tonight to add to the bare outline I have given.

Brigadier N. Hamilton Fairley: Mr. President, ladies and gentlemen, I had the privilege of visiting Dr. MACKIE's experimental typhus mission at Myitkyna—one could not fail to be impressed with the scope and detailed nature of the investigations being undertaken there. It has been very fascinating to listen to the clear-cut conclusions which have followed from this work, and I think there can be no doubt whatever in anyone's mind that *Trombicula debreuxi* is the vector in this region. Dr. MELLANBY referred to researches with dibutyl and dimethyl phthalate as a larval miticide in the Australian military forces. The first demonstration of the effectiveness of dimethyl phthalate against mites was made in the United States. The Orlando group of entomologists showed that dimethyl phthalate was the best protective there was against mites—they also showed that it was a very effective repellent as far as culicine mosquitoes were concerned. Dibutyl phthalate, on the other hand, had been found to be a less satisfactory mosquito repellent than dimethyl-phthalate by these observers, but no data regarding its effectiveness against

mites were available. When I was there with Dr ADRIEN ALBERT in September, 1942, these Orlando results were made available to us, and knowing that we had no efficient mosquito repellent and that we had to face the two problems of malaria and scrub typhus in the South-West Pacific, we immediately cabled the DIRECTOR GENERAL OF MEDICAL SERVICES advising that dimethyl phthalate should be manufactured in Australia and adopted in the armed forces.

About this time Australian Army entomologists commenced their investigations on these two substances. The effectiveness of dimethyl phthalate as a mosquito repellent was confirmed and its value against the Australian and New Guinea anopheline vectors of malaria clearly demonstrated. McCULLOCH investigated the action of both dimethyl and dibutyl phthalate against larval mites, and found them both equally effective miticides. He also conclusively demonstrated that dibutyl phthalate was better retained in clothing than dimethyl phthalate, persisting up to as many as seven washings. In Australia large-scale manufacture of dimethyl phthalate was commenced in January, 1943, that of dibutyl phthalate some months later. The large-scale use of these products was adopted by the Australian military forces in 1943, and their experience was much greater than that obtaining in either British or U S A troops, since neither of these phthalates was employed by them in the field until a much later date. There can be little doubt that where it was effectively applied in the manner shown in the excellent film prepared by Dr MELLANBY dibutyl phthalate proved exceedingly efficacious in preventing mite typhus in jungle warfare.

Dr A. Felix said the report presented by Colonel MACKIE contained a number of observations of interest to the "specialist," but he would comment on only two points that are of immediate practical importance to all who have come into contact with the disease or may have to do so in the future. The first point is the question of seasonal distribution. Before the outbreak of the last war it was known that tsutsugamushi in Japan had a definite seasonal incidence. The disease used to be called Japanese river-fever or flood-fever. It was known that with the cessation of the flooding the areas that had been inundated became infested with mice which had fled before the waters, and that the mice were mite infested. In Malaya, on the other hand, the disease occurred throughout the year, as was clearly shown by FLETCHER and LEWTHWAITE, whose observations covered a period of more than 15 years. The investigations on typhus of the OXK type in India, which were summarized by BOYD in 1935, were not extensive enough to allow of any definite conclusion. Now Colonel MACKIE and his colleagues report that the incidence of the disease among the American and Chinese troops operating on the Burma front was not truly seasonal, whereas the figures from the British Army in Burma indicate a definite seasonal distribution related to the monsoon. Colonel MACKIE considered the data specified in his Table V as being of particular

occurred in the autumn of 1943. This concerned some eighty cases around Mandalay.

When one tries to correlate the incidence of scrub typhus in South East Asia—I mean primarily the Indo-Burma border—with the concurrent operations, the available data are not perhaps sufficient for us to do so as accurately as Colonel MACKIE has done. But generally speaking one can say the operational season was essentially the dry weather—that is the first half of the year. That, after all, was the time when operations were more practicable. In 1943 they were mainly confined to patrol activities on the Central Front, but in 1944 and 1945 some of the heaviest fighting took place during the first half of the year.

It was in March, 1944 that three divisions fought their way back to the Imphal Plain along tracks where before and since large numbers of cases of typhus have occurred, yet the incidence at this time is seen to be negligible (see Graph 2). Nor did it begin to rise until troops moved out of Imphal over these very same tracks after the siege was raised the following May. In 1945 very heavy fighting was taking place during February and March in Central and Southern Burma when our troops were advancing on Mandalay. The incidence of typhus is again seen to be low but in the latter part of the year it rose steeply.

It was difficult to avoid the conclusion that the marked change in climate which occurs in Burma was one of the factors that influenced the number of cases and we came to regard the second half of the year as the typhus season as opposed to the first half which was known as the "shooting season."

I am very sorry that Dr. LEWTHWAITE and Lieut.-Colonel AUDY of the scrub typhus research teams set up in South-East Asia Command, are not here tonight to add to the bare outline I have given.

Brigadier N. Hamilton Fairley. Mr. President, ladies and gentlemen, I had the privilege of visiting Dr. MACKIE's experimental typhus mission at Myitkyina. One could not fail to be impressed with the scope and detailed nature of the investigations being undertaken there. It has been very fascinating to listen to the clear-cut conclusions which have followed from this work, and I think there can be no doubt whatever in anyone's mind that *Trombicula deliensis* is the vector in this region. Dr. MELLANBY referred to researches with dibutyl and dimethyl phthalate as a larval miticide in the Australian military forces. The first demonstration of the effectiveness of dimethyl-phthalate against mites was made in the United States. The Orlando group of entomologists showed that dimethyl phthalate was the best protective there was against mites. They also showed that it was a very effective repellent as far as culicine mosquitoes were concerned. Dibutyl phthalate, on the other hand, had been found to be a less satisfactory mosquito repellent than dimethyl phthalate by these observers, but no data regarding its effectiveness against



significance, because they were derived from the histories of a number of camp sites which were under observation for more than a year. The figures would perhaps be more convincing if Colonel MACKIE were in a position to state not only the number of cases in the individual camps but also the number of personnel in occupation of each camp at the material time. On the other hand the graph shown by Colonel SAYERS is based on the total incidence of the disease in the British Army in Burma comprising many hundreds of thousands of troops and a more detailed analysis of these overall figures might possibly show a somewhat different picture.

The other point of general interest is the experimental proof of transovarial transmission of rickettsiae in the mite and the members of the United States of America Typhus Commission are to be congratulated on this achievement. The conclusion that the mite is to be regarded as both the vector and an important reservoir of the infection should, however, not detract from the importance of the wild rat as a reservoir. This, of course is a most important point in the choice of preventive measures, whether these should be directed against the mite or against the rat. An analogous relationship exists between vector and reservoir in the case of Rocky Mountain spotted fever. Hereditary transmission of rickettsiae has long been known in the tick, the vector of Rocky Mountain spotted fever nevertheless, it is thought that the maintenance of the infection in nature depends on the presence of a susceptible animal which serves as a reservoir. This view is held by Dr. R. R. PARKER, the outstanding authority on Rocky Mountain spotted fever whom I had the privilege of visiting recently at the Rocky Mountain Laboratory in Hamilton Montana. According to PARKER, although a female tick lays several thousand eggs, all of which may hatch, only a few individuals escape the many risks of life to complete the full cycle and pass on the rickettsiae to the next generation. In the case of scrub typhus, it would appear that wild rats are an important reservoir of the infection, if not the most important one. "Anti mite" measures have been of great importance under conditions of active warfare, but in peace time anti rat measures may possibly prove to be of even greater value.

Dr. R. H. P. Clark: Mr. President, speaking as one with a slight knowledge of the ground covered I suggest that one explanation of the conflicting findings as to seasonal incidence lies in varying climatic conditions. The Ledo Road could hardly afford a greater contrast to the arid district of Burma south of Mandalay and east of the Yoma watershed and I suggest that the discrepancy might be resolved on these lines.

Dr. C. Hollins: I have never been to Burma, and have never seen a case of tsutsugamushi fever but I have recently seen two epidemics of M19 typhus in Nigeria, and I would like to ask Colonel MACKIE two questions. These

epidemics were transmitted by lice, and I would like to know whether he tried to transmit scrub typhus by lice? Another thing is that I would like to hear something of the clinical side of the disease. The cases that occurred in Nigeria were exactly like encephalitis lethargica, and I came to the conclusion that one of the quickest aids to diagnosis was spinal puncture. Did Colonel MACKIE find the same in tsutsugamushi fever?

Colonel Mackie (in reply) Since the primary mission of the India Burma Field Headquarters was limited to the epidemiology of scrub typhus relatively little attention was devoted to the clinical features of disease. Such studies, however, were conducted by other groups, notably by members of the staff of the 20th General Hospital at Ledo, Assam, and have been reported elsewhere by them.

I regret that we have no information concerning the incidence of scrub typhus among the refugees reaching Ledo in the course of the evacuation of Burma. In view of the distribution of the disease observed in the course of the subsequent military operations, one would anticipate that they must have suffered from the infection.

It is abundantly evident that there is no racial immunity to scrub typhus since the infection was observed to occur in many of the peoples comprising the Forces in South-East Asia. A number of cases from the labour battalions working along the Stilwell Road were treated at the 94th Indian General Hospital in Ledo. This same institution likewise reported several cases from the Kachin Rifles. Specific information concerning its occurrence under ordinary conditions among the Kachin and Nagas was unobtainable. This is not unexpected in view of the remoteness of their villages and the limited contact with Europeans and with occidental medicine. It is of interest, likewise, that British officers and civilians long resident in Assam and Upper Burma before the war were wholly unfamiliar with the disease and the belief was widespread that it was new to the area and introduced by the Japs. Lack of a workable complement fixation technique made it impossible to investigate possible specific immunity among the indigenous population, since a positive Weil-Felix reaction does not persist long into convalescence. One is left with the impression that the troop concentration and the activities of the individual soldier created conditions peculiarly favourable to the disease.

It is impossible to give a wholly satisfactory answer to the question concerning incidence of the various rickettsial diseases. We were informed that during the early stages of the American participation considerable difficulty was experienced with the Weil-Felix reaction because of lack of satisfactory antigens. Subsequently when this had been corrected the diagnostic difficulties were largely resolved. There remained, however, a small proportion of cases giving atypical serological reactions in the presence of a characteristic clinical picture. In addition to scrub typhus, a number of cases of classical louse-borne typhus



occurred in Chinese troops recently transported from China by air. Our evidence does not indicate that other members of the rickettsial group of fevers occurred in the areas under consideration.

It is recognized that the significance of Table V "Incidence of Scrub Typhus in Chinese Army Camp Sites along the Stilwell Road," would be enhanced by enumeration of the population at risk and the calculation of attack rates. Serious efforts were made to obtain the necessary basic data. While reasonably accurate figures for unit strengths during the period of the outbreak in the spring of 1945 were obtainable, the records prior to that time were grossly incomplete.

The conclusion which we reached from the studies of case incidence in the United States and Chinese armies that "there appeared to be no strict seasonal distribution of the disease in man and it may be acquired throughout the year" is not in serious conflict with the definite seasonal distribution observed in the 14th Army. The significant observations are, I believe, that the disease may be acquired at any season and that the activity of the individual is an important factor determining the magnitude of the hazard. The small size of the U.S. combat force in contrast to the strength of the 14th Army tends to make statistical comparison invalid. Such lack of conflict is further supported by the evidence incriminating *T. deliensis* as the vector and by the finding of this mite in greatest abundance at the beginning and the end of the monsoon season.

The interpretation of transovarial transmission of the rickettsiae in *T. deliensis* as indicating that the mite must be regarded as a reservoir of infection is not intended to exclude the importance of rodent reservoirs. Both must be considered in any programme for the control of scrub typhus. In areas where the disease is known to be endemic both anti-rat campaigns and strict individual prophylaxis should be utilized if the disease incidence is to be kept at a minimum. As has been pointed out, the method of individual protection against the mites so well shown by the British Army training film has been proved beyond question. Scrub typhus disappeared from units in which this procedure was properly carried out.

## COMMUNICATIONS.

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### THE DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN CENTRAL AND EAST AFRICA \*

BY

A F MAHAFFY,  
K. C SMITHBURN,

AND

T P HUGHES

*From the Yellow Fever Research Institute, Entebbe, Uganda †*

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An investigation of the geographical distribution of immunity to yellow fever in man was begun by the International Health Division of The Rockefeller Foundation in 1931, and the results of a general survey in North, East and South Africa were published by SAWYER and WHITMAN (1936) In that report the

\* All sera from Tanganyika Territory, as well as many of those from Uganda, the Belgian Congo, Kenya, the Anglo-Egyptian Sudan, Eritrea and Somalia were collected by members of the Institute staff, who included, in addition to the authors, Dr A W BURKE and Dr H R JACOBS In this work we enjoyed the close co-operation and assistance of the medical and administrative authorities For the remainder of the collections we are indebted to the Directors of Medical Services and their assistants in the territories concerned For the preparation of the maps we are indebted to FLORENCE SMITHBURN In addition to the authors, Drs J H PAUL, H R JACOBS and K GOODNER, members of the Institute staff, participated in carrying out the tests in the laboratory

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findings of surveys previously carried out in West Africa were reviewed and the approximate boundaries of the continental zone of immunity to yellow fever were indicated.

Since the establishment of the Yellow Fever Research Institute in Entebbe the survey of Central and East Africa has been intensified and extended in an attempt to delimit more accurately the zone where yellow fever has occurred. The study was commenced in 1937 and all results obtained up to the end of 1943 are included in this report. During that period 10,274 sera from residents of ten countries were examined. In tabulating the results all tests done at the Institute in Entebbe have been included, although some of those with sera from Uganda, the Anglo-Egyptian Sudan and the Belgian Congo have already been published (KIRK, 1941 FINDLAY *et al.* 1941 HUGHES *et al.*, 1941 LINGGÖIS, 1944).

In addition to the general survey intensified studies were made in restricted areas for special purposes, *viz.*, the investigation of fatal cases of yellow fever (Bondo and Yatolesma in the Congo Kitale and Langata in Kenya, and Torit in the Sudan), and special investigations in epidemiology (Bwamba, Uganda, and Watalinga, Congo). Results of these studies are included with those of the general survey.

The intraperitoneal protection test was employed throughout. The methods used in testing the sera and in the interpretation of the results were essentially the same as those originally described by SAWYER and LLOYD (1931). A 20 per cent. suspension of virus-containing mouse brain was employed in the earlier tests, but this was later reduced to 10 per cent., and since the beginning of 1943 a 1 per cent. suspension has been used. A satisfactory test in baby mice was used during the year 1943 for sera available in less than 3 ml. amounts. The technique of the test in baby mice as well as the experimental evidence which resulted in the adoption of the 1 per cent. virus suspension has been described by one of us (SMITHBURN 1945).

The results of the tests have been tabulated by countries, and maps have been prepared showing the location of the places included in the survey (Tables I to XI and Figs. 2 to 10). Sera which gave inconclusive, toxic or unsatisfactory results were eliminated and are not included in the tabulations. Places where immune persons were found are indicated on the maps by solid circles, whereas a blank circle denotes absence of immunity. Data concerning the number and age group of persons examined, as well as the number and percentage of immunes, are given in the tables. Percentages are not shown where the number of persons examined was less than ten. Fig. 1 is a general outline map of Africa showing the relationship to one another of the areas included in Figs. 2 to 8. This map also shows the approximate boundary of the zone in Africa where immunity to yellow fever has been found in the survey here reported or in previous surveys.

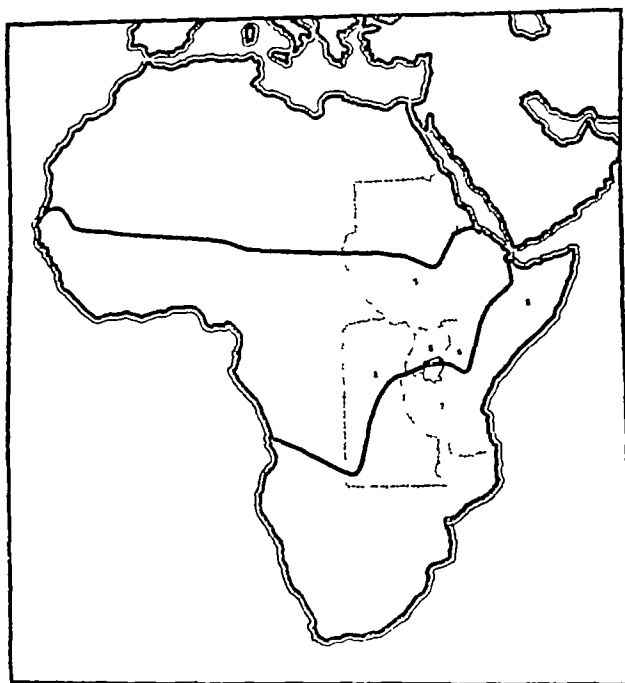


FIG 1 AFRICA, SHOWING TERRITORIES COVERED BY THE IMMUNITY SURVEY

The numbers refer to the corresponding large scale maps of these areas which follow in the text. The heavy solid line indicates the approximate boundary of the area within which immunity to yellow fever has been found.

## RESULTS

### *Belgian Congo*

The results of tests with 1,626 sera from residents of the Belgian Congo are given in Table I, p 60, Fig 2, p 64, shows where the collections were made. Some of these specimens were collected in connection with field studies carried out in two areas in Stanleyville Province and in the Watalinga district of Costermansville Province. The remainder came from that portion of the eastern border of the country which lies south of Watalinga, including the mandated territory of Ruanda-Urundi.

Following the occurrence of a fatal case of yellow fever in the Bondo district of Stanleyville Province in 1937, a field investigation was initiated and 589 sera were collected in eighteen localities in 1940, with the generous collaboration of Dr P LIEGEOIS. The donors were all children, and immune persons were found in ten of the places sampled. The percentage of immunes was low in all except the Kende area, which lies near the border of French Equatorial Africa. Immunity in the adjoining area in French territory had previously been shown to be high (BEEUWKES *et al*, 1934), and at one place (Zemio) it reached 95 per cent in adults and 36 per cent in children.

TABLE I.  
BELGIAN CONGO.

Place.	Year of survey	Number examined.	Number immune.	Percent age immune.	Age group 0-14 years.			Age group 15 years & over		
					Number examined.	Number immune.	Percent age immune.	Number examined.	Number immune.	Percent age immune.
Kinshasa Province										
Iketa	1940	60	4	6.7	24	1	4.2	36	3	8.3
Ipi	40	7	0	—	7	0	—			
Iapi	40	26	0	0.0	26	0	0.0			
Ihi	40	88	0	0.0	88	0	0.0			
Iondo	40	28	2	5.6	28	2	5.6			
Iona	40	23	0	0.0	23	0	0.0			
Iyaba	40	12	0	0.0	12	0	0.0			
Iongobe	40	24	2	8.3	24	2	8.3			
Iambo	40	31	0	0.0	31	0	0.0			
Iu	40	19	0	0.0	19	0	0.0			
Iuvu	41	4	0	0.0	11	0	0.0	12	0	0.0
Ivenda	40	19	0	0.0	19	0	0.0			
Iagumbo	40	29	3	7.7	29	3	7.7			
Iokono	40	30	0	0.0	30	0	0.0			
Iosaga	40	82	3	3.6	82	3	3.6			
Iungba	40	17	1	5.9	17	1	5.9			
Iaboni	40	16	0	0.0	16	0	0.0			
Ioa	40	59	0	0.0	59	0	0.0			
Iilaba	40	19	2	10.5	19	2	10.5			
Ivotolama	41	81	4	7.8	79	1	3.8	23	3	13.0
Iabala	41	48	8	11.1	18	0	0.0	27	8	13.3
Ialilo	41	16	4	25.0	2	1	—	14	3	21.4
Iasendu	41	27	3	11.1	12	0	0.0	14	3	21.4
Iacotondja	41	23	3	13.0	12	0	0.0	10	3	30.0
Iasongo	41	25	8	32.0	12	4	33.3	13	1	7.7
Ialikanda	41	27	2	7.4	12	0	0.0	15	2	13.3
Iasonga	41	58	10	17.2	26	1	3.8	26	9	34.6
Iambaba	40	35	0	0.0	35	0	0.0			
Ierre	40	17	0	0.0	17	0	0.0			
Iorasi	40	83	1	1.2	82	1	1.2			
Kisumu Province										
Iamf	41	29	0	0.0	15	0	0.0	14	0	0.0
Iobeta	41	29	7	24.1	16	0	0.0	13	7	53.8
Iygmies	41	13	0	0.0	4	0	—	9	0	—
Iewa	41	18	3	16.7	18	3	16.7			
Iubane	41	27	2	7.4	12	0	0.0	15	2	13.3
Iygmies	41	25	0	0.0	0	0	—	19	0	0.0

TABLE I—continued

Place	Year of survey	Number examined	Number immune	Percentage immune	Age group 0-14 years			Age group 15 years & over		
					Number examined	Number immune	Percentage immune	Number examined	Number immune	Percentage immune
Kamango	1941	26	1	3.8	26	1	3.8			
Kasindi	'41	26	0	0.0	12	0	0.0	14	0	0.0
Kikango	'41	14	0	0.0	14	0	0.0			
Kizanzamba	'41	14	4	28.6	14	4	28.6			
Lubero	'41	36	0	0.0	32	0	0.0	4	0	—
Mambopia	'41	10	3	15.8	19	3	15.8			
Molopia	'41	11	5	45.5	11	5	45.5			
Mueta	'41	25	2	8.0	11	0	0.0	14	2	14.3
" pygmies	'41	26	1	3.8	9	0	—	17	1	5.9
Muntunduluku	'41	14	1	7.1	14	1	7.1			
Mutwanga	'41	31	0	0.0	16	0	0.0	15	0	0.0
Rutshuru	'41	23	0	0.0	23	0	0.0			
Vuhovi	'41	25	0	0.0	12	0	0.0	13	0	0.0
Weyana	'41	26	0	0.0	11	0	0.0	15	0	0.0
Elizabethville Province										
Kabunda	'41	23	0	0.0	18	0	0.0	5	0	—
Kasenga	'41	27	0	0.0	27	0	0.0			
Kiniana	'41	22	0	0.0	15	0	0.0	7	0	—
Pweto	'41	14	0	0.0	14	0	0.0			
Sakania	'41	20	0	0.0	20	0	0.0			
Ruanda-Urundi										
Brumba	'41	13	0	0.0	13	0	0.0			
Muhinga	'41	20	0	0.0	17	0	0.0	3	0	—
Nyanga	'41	8	0	—	8	0	—			
Ruhengeri	'41	17	0	0.0	17	0	0.0			
Ruvigi	'41	11	0	0.0	9	0	—	0	0	—
Total		1 626	105	6.5	1 248	61	4.9	378	44	11.6

No cases of yellow fever were observed during the field study in the Bondo area in 1940, but a viscerotomy service begun at that time has since revealed the presence of the disease in the area on several occasions.

The sera from the Yatoema area near Stanleyville were collected as part of an investigation of a fatal case of yellow fever in a European which occurred there in November 1940. An intensive study of the area over a period of 6 months failed to reveal any further cases or any definite evidence that other



cases had occurred in the immediate past. The immunity survey which was carried out in January 1941 showed that 5.6 per cent. of children and 17.4 per cent. of adults resident in the area were immune to yellow fever. Since no previous survey had been made in the district it is not possible to determine to what extent these findings were due to recent activity of the virus. The available evidence however suggests that the 1940 outbreak was very limited in extent.

The Watalinga district is located in the valley of the Semliki river immediately west of Bwamba County Uganda. Following the outbreak of yellow fever in Bwamba in 1941 (MAHAFFY *et al.*, 1942), sera for examination in the protection test were collected from residents of the Watalinga district, as well as from persons living in forest villages on the high ground west of the Semliki valley in order to learn whether the population on the Congo side of the Lamira river had been affected. The results indicated recent activity of the disease in the population living in or near the forest in Watalinga, whereas the sera from children living in the forest west of the valley gave completely negative results. These findings suggest that the epidemic which is known to have occurred in Bwamba in 1941 was confined to a limited area in the valley of the Semliki river.

The sera from the eastern border of the Belgian Congo south of the Watalinga district, including those from Ruanda-Urundi were all non-protective, a finding which is of some interest considering the results obtained in Northern Rhodesia.

Apart from the special studies referred to above, carried out in the Belgian Congo, the purpose of our immunity survey in that country was primarily to cover the portion of its eastern border which lies south of Stanleyville Province. An immunity survey in the rest of the country is being done by Dr P. LIEZONIS, Director of the laboratory in Stanleyville, some of whose results have already been published (LIEZONIS, 1944).

#### *Northern Rhodesia.*

During the year 1941 110 sera from three localities in the Northern Province of Northern Rhodesia were tested and all were without protective properties against yellow fever virus (Table II and Fig. 2).

In September 1943 a patient, with symptoms suggesting yellow fever was admitted to hospital in Balovale, which is situated on the Zambezi river in the north-west corner of the country and near the Angola border. The case was not proven to be yellow fever since all that is known is that the patient's serum taken during the later stages of the illness was protective. In view of the fact that the patient had not travelled and must therefore have been immunized in the district, a survey was undertaken to determine to what extent the population had been affected. Sera from 178 persons who were resident in Balovale and the surrounding area were tested and fifteen, or 8.5 per cent.

TABLE II  
NORTHERN RHODESIA

Place.	Year of survey	Number examined	Number immune	Percentage immune	Age group 0-14 years			Age group 15 years & over		
					Number examined	Number immune	Percentage immune	Number examined	Number immune	Percentage immune
Northern Province										
Abercorn	'41	46	0	0.0	3	0	—	43	0	0.0
Fort Rosebery	'41	47	0	0.0	27	0	0.0	20	0	0.0
Mbereshi	'41	17	0	0.0	1	0	—	16	0	0.0
Kaonda-Lunda Province										
Balovale (District)	'43	176	15	8.5	46	2	4.3	130	13	10.0
Kalene Hill	'42	12	0	0.0	2	0	—	10	0	0.0
Total		298	15	5.0	79	2	2.5	219	13	5.9

of them were found to be immune to yellow fever. The youngest immune donor was a child of 6 years. The survey in Northern Rhodesia is being continued in an attempt to delimit the area of recent infection. The results to date indicate that the disease has been present in the Balovale district within the last 6 years. This is an important finding in that yellow fever has not previously been shown to have occurred in Africa south of 10° S latitude. In view of the absence of immunity in that portion of the Congo north east of Balovale, these results point to the desirability of extending the survey westward into Angola.

#### *Anglo-Egyptian Sudan*

The results of tests with sera from the Sudan are given in Table III and Fig 3 (p 67). The total listed in the table is 1,262 but, as mentioned above, many of these have already been reported (KIRK, 1941, FINDLAY *et al*, 1941). The remaining results are those with sera collected in Equatoria Province in 1938 and 1942 and in the southern Fung area in 1942. In a survey of the Fung area in 1937-38 (FINDLAY *et al*, 1941), nineteen of 132 persons were found to be immune to yellow fever. Donors included both children and adults, but no immune children were found. Since then we have carried out additional studies in the southern Fung area and have shown that yellow fever has recently occurred as far east as the Abyssinian border. The specimens were collected by Dr R. KIRK, of the Sudan Medical Service, who took special care to select donors who had always lived in the area being sampled. In these circumstances

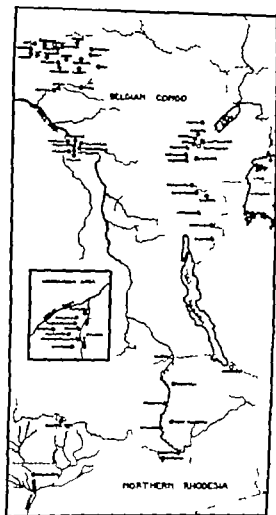


FIG. 2. DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN THE BELGI CONGO VS NORTHERN RHODESIA.

Solid circles denote immunity; black circles denote no immunity. Findings for the Watafinga area, indicated by the small square, are given in the inset map.

the finding of immune children in places on or near the border should be regarded as significant even though the number is small. Unfortunately we have not so far been able to obtain sera from the area east of the Fung Abyssinian border.

The results of the survey in 1938 of that portion of Equatoria Province which lies west of the Nile confirmed earlier findings and indicate that yellow fever has occurred in, and has been widely distributed throughout, this area.

TABLE III  
ANGLO-EGYPTIAN SUDAN

Place	Year of survey	Number examined	Number immune	Percent-age immune	Age group 0-14 years			Age group 15 years & over		
					Num-ber exam-ined	Number immune	Percent-age immune	Num-ber exam-ined	Number immune	Percent-age immune
Equatoria Province										
Aweil	'38	28	5	17.9	27	4	14.8	1	1	—
Gognal	'38	29	3	10.3	29	3	10.3			
Kojo Kaji	'39	93	0	0.0	56	0	0.0	37	0	0.0
Kapoeta	'41	25	1	4.0	8	1	—	17	0	0.0
Katire	'42	32	8	25.0				32	8	25.0
Kolya	'38	25	1	4.0	25	1	4.0			
Maridi	'38	25	10	40.0	14	3	21.4	11	7	63.6
Runtch	'38	26	5	19.2	20	3	15.0	6	2	—
Tont	'41	27	1	3.7	11	0	0.0	17	1	5.9
"	'42	60	3	5.0	31	3	9.7	29	0	0.0
Woway	'38	25	1	4.0	25	1	4.0			
Yei	'38	38	1	2.6	18	0	0.0	20	1	5.0
Yubo	'38	25	0	0.0	25	0	0.0			
Kordofan Province										
Bara	'41	27	0	0.0	11	0	0.0	16	0	0.0
El Obeid	'41	10	0	0.0				10	0	0.0
Heiban	'41	44	29	65.9	14	9	64.3	30	20	66.7
Otoro Hills	'41	13	9	69.2	8	5	—	5	4	—
Rahad	'41	26	1	3.8	3	0	—	23	1	4.3
Rashad	'41	20	0	0.0	6	0	—	14	0	0.0
Tagoi	'41	98	0	0.0	39	0	0.0	59	0	0.0
Tira Hills	'41	28	24	85.7	4	3	—	24	21	87.5
Jm Burumbeita	'41	20	1	5.0	3	0	—	17	1	5.9
Jm Kas	'41	13	0	0.0	1	0	—	12	0	0.0
Blue Nile Province										
Abuldugu	'42	24	11	45.8	3	0	—	21	11	52.4
Abungaru	'42	22	1	4.5	9	0	—	13	1	7.7
Alwara	'42	30	3	10.0	5	0	—	25	3	12.0
Boing	'42	26	6	23.1	4	1	—	22	5	22.7
Ed Dueim	'41	12	0	0.0	3	0	—	9	0	—
Fashishoya	'41	12	0	0.0	3	0	—	9	0	—
Fazoughi	'42	34	13	38.2	9	2	—	25	11	44.0
Febenait	'42	14	3	21.4	7	0	—	7	3	—
Gulli	'41	15	0	0.0	4	0	—	11	0	0.0
Hebelein	'41	13	0	0.0	8	0	—	5	0	—
Hebel Baak	'42	22	1	4.5	11	0	0.0	11	1	9.1
Hebel Maghaja	'42	11	4	36.4	6	0	—	5	4	—

TABLE III—continued.

Place.	Year of survey	Number examined.	Number immune.	Percent age immune.	Age group 0-14 years.			Age group 15 years & over		
					Number examined.	Number immune.	Percent age immune.	Number examined.	Number immune.	Percent age immune.
Jebel Mufwa ...	42	28	7	25.0	13	0	0.0	15	7	46.7
Khor Agol ...	41	15	0	0.0	6	0	—	9	0	—
Qarema ...	41	15	0	0.0	3	0	—	12	0	0.0
Shabasha ...	41	12	0	0.0	3	0	—	9	0	—
Soda ...	42	20	11	55.0	7	0	—	19	11	57.9
Wadega ...	42	29	7	24.1	13	0	0.0	16	7	43.8
Wako ...	42	24	3	12.5	11	1	9.1	13	2	15.4
Kassala Province										
Doka ...	41	10	1	10.0	1	0	—	9	1	—
Godaref ...	41	23	0	0.0	10	0	0.0	13	0	0.0
Gharagana ...	41	14	0	0.0	10	0	0.0	4	0	—
Kassala ...	41	10	0	0.0	4	0	—	6	0	—
Showak ...	41	14	0	0.0	9	0	—	5	0	—
Sofi ...	41	14	0	0.0	4	0	—	10	0	0.0
Red Sea (atoral)										
Aqiq ...	41	4	0	—	2	0	—	2	0	—
Port Sudan ...	42	13	0	0.0	7	0	—	6	0	—
Boakdi ...	41	11	0	0.0	10	0	0.0	1	0	—
Tokar ...	41	8	0	—	5	0	—	3	0	—
Total		1,253	174	13.9	563	40	7.0	694	134	19.2

Collections east of the Nile in Equatoria Province were made during an investigation of a fatal case of yellow fever in a European, which occurred at Torit in October 1942. The disease had not previously been recognized in this area and in the 1941 survey only two immune individuals were found—one in Torit and one in Kapoeta. The Torit immune was an adult of 35 years and was the only one in a group of twenty-eight persons ranging in age from 9 to 45 years. Following the occurrence of the fatal case in 1942, sixty sera—twenty nine from children and thirty-one from adults—were obtained from residents of Torit, all of whom stated that they had never travelled outside the district. Three of the children were found to be immune, but the sera from adults were all non-protective. Although the finding of immune children suggests that there may have been an outbreak in Torit since the earlier survey it is apparent that only a small percentage of the population was immunized. The fatal case in the European was the only one observed. This man had not

been away from Torit for at least 4 months prior to the onset of the illness, and there can be no doubt that he was infected there

In an attempt to discover a possible source of infection of this apparently isolated case a visit was made to Katire in the Imatong Mountains about 35 miles south east of Torit. These mountains, which have peaks reaching an altitude of 10,000 feet, are heavily wooded and are inhabited up to 4,000 feet by the Imatong tribe. Blood specimens were taken from thirty-two adult

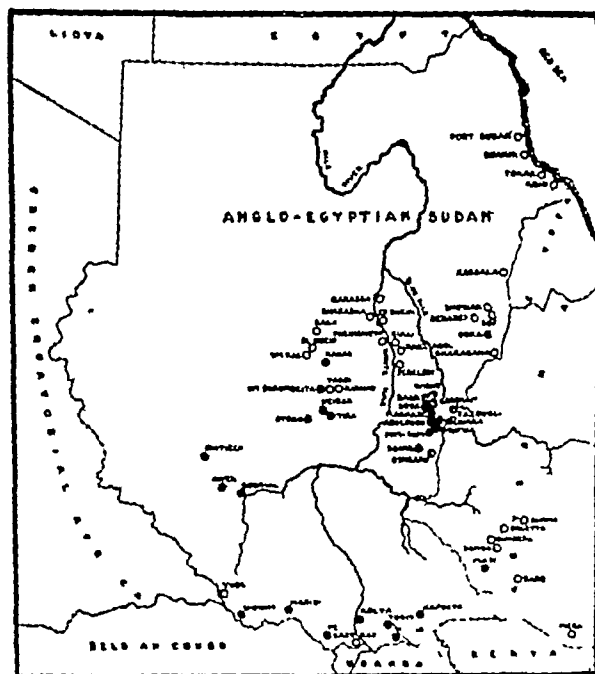


FIG 3 DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN THE ANGLO EGYPTIAN SUDAN AND ABYSSINIA.

Solid circles denote immunity, blank circles denote no immunity

members of this tribe, who stated that they had never travelled more than a few miles from their homes in the hills. Eight, or 25 per cent of them, were found to be immune to yellow fever. This is an interesting finding, the significance of which cannot be fully assessed until further studies have been made. It is, however, worth noting that this high degree of immunity to yellow fever was found in an area which, although hitherto isolated, has recently come into close contact with Torit through developments in the timber industry. A reasonably good motor road now connects Katire, the centre of this industry, with Torit, and lorries are constantly passing between these communities. If, as seems likely, it should be found that there is a focus of the disease in the

Imstong Mountains similar to that known to exist in the valley of the Semliki river in western Uganda, we have a possible explanation of the source of the infection which appeared in Torit.

### *Abyssinia.*

The survey in Abyssinia has been limited to the south western portion of the country where 223 sera from seven places have been examined. Only one protective serum was found. This was from a person who normally lived in Maji but who was in a refugee camp in Kenya at the time the serum was obtained. Table IV includes the results with eighty-eight sera from the Gimma area collected early in 1944. All were non-protective. These findings suggest that yellow fever is not endemic in south western Abyssinia, but nothing is known at present of the situation in the greater part of the country.

TABLE IV

ABYSSINIA.

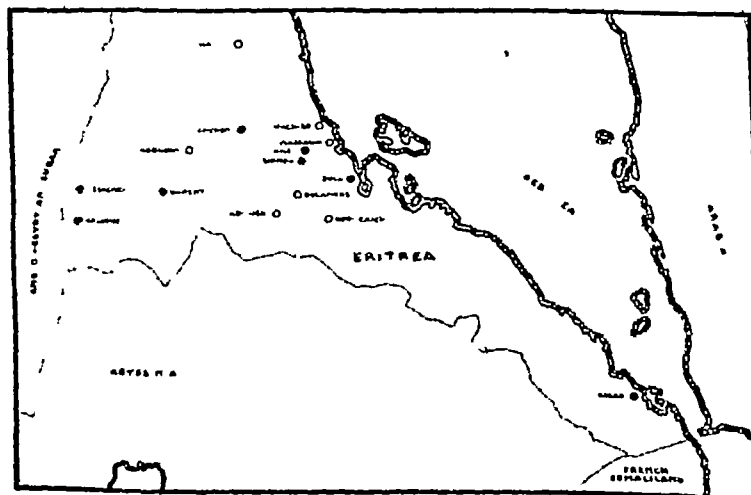
Place.	Year of survey	Number examined.	Number immune.	Percent age immune.	Age group 0-14 years.			Age group 15 years & over		
					Number examined	Number immune.	Percent age immune.	Number examined.	Number immune.	Percent age immune.
Bako	41	23	0	0.0				23	0	0.0
Baleta	44	27	0	0.0				27	0	0.0
Bonga	44	23	0	0.0				22	0	0.0
Dimbets ...	44	28	0	0.0				26	0	0.0
Gimma	44	14	0	0.0				14	0	0.0
Maji	41	51	1	2.0				51	1	2.0
Mega ...	42	51	0	0.0				51	0	0.0
Total		223	1	0.4				223	1	0.4

### *Eritrea.*

Table V gives the results of tests with 528 sera collected in fifteen localities in Eritrea. As shown in Fig. 4 the places selected for sampling extend across the country from Assab Zulu and Massawa on the Red Sea coast to Tsemene near the Sudan border. The results indicate that yellow fever has recently occurred in Eritrea. There is no evidence that the population living on the high central plateau has been affected, but immune individuals—both children and adults—were found in the coastal area and on the western plains. The finding of immune children in three localities on or near the Red Sea coast clearly indicates that the disease has been active in this area within the last 15

TABLE V  
ERITREA

Place.	Year of survey	Number examined	Number immune.	Percentage immune	Age group 0-14 years			Age group 15 years & over		
					Number examined	Number immune	Percentage immune	Number examined	Number immune	Percentage immune
Addi Caeh	'42	26	0	0 0	1	0	—	25	0	0 0
Adi Ugru	'42-'43	50	0	0 0	33	0	0 0	17	0	0 0
Agordat	'42-'43	54	0	0 0	25	0	0 0	29	0	0 0
Ailet	'43	26	4	15 4	22	2	9 1	4	2	—
Assab	'42-'43	48	3	6 3	31	3	9 7	17	0	0 0
Barentu	'42-'43	53	3	5 7	25	1	4 0	28	2	7 1
Cheren	'42-'43	50	2	4 0	30	1	3 3	20	1	5 0
Decamere	'42	25	0	0 0				25	0	0 0
Galouge	'43	26	1	3 8	26	1	3 8			
Ghinda	'42	19	1	5 3	2	0	—	17	1	5 9
Massawa	'42	30	0	0 0				30	0	0 0
Nacfa	'43	25	0	0 0	25	0	0 0			
Tessenei	'42-'43	51	3	5 9	25	2	8 0	26	1	3 8
Uachiro	'43	24	0	0 0	23	0	0 0	1	0	—
Zula	'43	19	4	21 1	19	4	21 1			
Total		526	21	4 0	287	14	4 9	239	7	2 9

FIG 4 DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN ERITREA  
Solid circles denote immunity, blank circles denote no immunity



years. This does not necessarily mean that there has been a recent eastward spread of the disease, since no previous surveys have been made in Entrea. It does mean, however that Entrea, including its coastal belt, should now be regarded as potentially infective—a point of the utmost importance, particularly to places such as India and the Orient which up to the present have been free of the disease.

### Somalia.

One hundred and forty-six sera from four places in Somalia were examined. The results are given in Table VI and Fig. 5. No immune persons were found in either of the two most important ports, Mogadishu and Kismayu, but one of twenty-seven sera from residents of Villagio was protective. The positive donor was a woman of at least 70 years of age, who demanded that her blood be taken and who stated very emphatically that she had never travelled. Little importance can be attached to this finding.

TABLE VI.  
SOMALIA.

Place.	Year of survey	Number examined.	Number known.	Percent age immune.	Age group 0-14 years.			Age group 15 years & over.		
					Number examined.	Number immune.	Percent age immune.	Number examined.	Number immune.	Percent age immune.
Daghabur	43	44	4	9.1	15	0	0.0	29	4	13.8
Kismayu	42	34	0	0.0				24	0	0.0
Mogadishu	42	51	0	0.0				51	0	0.0
Villagio	42	27	1	3.7				27	1	3.7
Total		146	5	3.4	15	0	0.0	131	5	3.8

The specimens from Daghabur were taken by Major G. W. A. Dick, R.A.M.C. following a report that a severe and often fatal illness of short duration and associated with fever and jaundice had been epidemic there in April and May 1943. Cases were not seen by anyone competent to make a clinical diagnosis nor was viscerotomy practised. In the absence of accurate and reliable information the nature of the illness remains obscure. It was probably not yellow fever. However the finding of four immunes in a group of twenty-nine adult residents of Daghabur indicates that yellow fever has occurred there at some time in the recent past. Further studies in western Somalia and eastern Abyssinia are indicated.

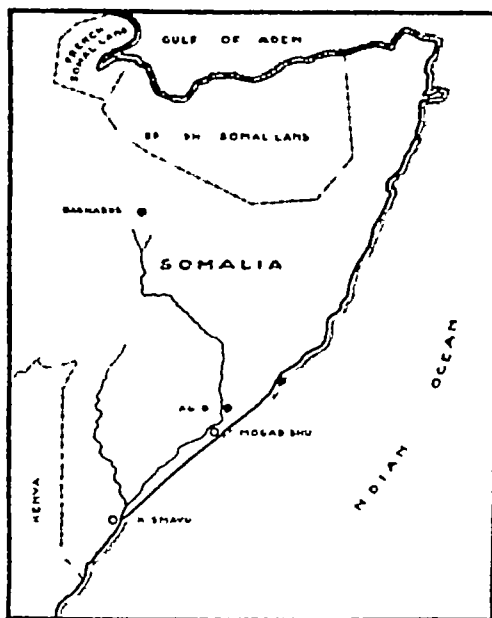


FIG 5 DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN SOMALIA  
Solid circles denote immunity, blank circles denote no immunity

### *Kenya*

Table VII gives the results of tests with 564 sera from Kenya, 338 of these came from north, central and south Kavirondo in Nyanza Province (Fig 6). A considerable number of localities, all of which were near the north east shore of Lake Victoria, were sampled. None of the sera gave protection.

The 166 specimens from the Rift Valley and North Frontier Provinces were tested as part of an investigation of a fatal case of yellow fever which occurred in Kitale in May, 1942. The disease had not previously been recognized in Kenya, but clinical history of this case, as far as it could be ascertained, was so suggestive of yellow fever that the medical attendant made strenuous and eventually successful efforts to obtain a specimen of liver, sections of which showed the characteristic lesions. The investigation revealed no evidence of other cases having occurred in the area, and 166 sera from individuals resident in Kitale and the surrounding district were without protective properties.

A second fatal case of yellow fever occurred in Kenya in November, 1943. The patient, an African soldier, died at Kisumu, but a study of his movements prior to death made it clear that infection must have occurred while he was living in an army camp near Nairobi. The camp was situated within a few hundred yards of the southern limit of the Langata forest, which the soldiers frequently visited. This is an indigenous forest of considerable extent which, on investigation, was found to be inhabited by about 300 squatters. As the forest seemed the most likely place in which to look for evidence of recent activity

TABLE VII.

KENYA.

Place.	Year of survey	Number examined.	Number immune.	Percent age immune.	Age group 0-14 years.			Age group 15 years & over.		
					Number examined.	Number immune.	Percent age immune.	Number examined.	Number immune.	Percent age immune.
Nyanza Province										
North Kavirondo	40-42	220	0	0.0	116	0	0.0	104	0	0.0
Central Kavirondo	40-42	107	0	0.0	27	0	0.0	80	0	0.0
South Kavirondo	40-42	11	0	0.0	2	0	—	9	0	—
Rift Valley Province										
Kitale	42	93	0	0.0	63	0	0.0	25	0	0.0
Endebess.	42	23	0	0.0	22	0	0.0			
North Frontier Province										
Kapenguria	42	40	0	0.0	19	0	0.0	21	0	0.0
Central Province										
Langata Forest	42	60	2	3.0	9	1	—	51	2	3.9
Total		564	2	0.4	274	1	0.3	290	2	0.7



FIG. 6. DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN KENYA.  
Solid circles denote immunity; blank circles denote no immunity.

of the virus, sixty sera were obtained from squatters and examined in the protection test. One of nine children and two of fifty-one adults were found to be immune to yellow fever. The movements of the three positive donors were carefully investigated and all were found to have been permanent residents in the forest since birth. The evidence indicates that yellow fever has been present in the Langata forest within recent years, and it is highly probable that the soldier dying of the disease in 1943 was infected there.

A better understanding of the epidemiological factors concerned is required before we can estimate the significance of the occurrence of these sporadic, or apparently sporadic, cases. They may result from the introduction of the virus from an outside source or, on the other hand, they may be due to the persistence of the virus in areas in which conditions are unfavourable to the spread of the infection. Further work, including animal studies in forested areas, may help to clarify the situation.

### *Tanganyika Territory*

The survey in Tanganyika Territory was limited to the western border, where 467 sera were collected in fifteen localities. The results are given in Table VIII, p 74. Fig 7 shows the location of places where specimens were taken. Only one serum was protective. The positive donor was a boy of 10 years resident in Ngara, near the Ruanda-Urundi border. This single positive is not regarded as significant, and the findings in western Tanganyika are interpreted as indicating freedom from infection during the lifetime of the present generation.

### *Zanzibar*

Sera from seventy-seven residents of Zanzibar were all non-protective (Table VIII, Fig 7).

### *Uganda*

In the Uganda Protectorate 5,085 sera from residents of fifty-six localities were examined. The results are given in Table IX. Apart from Bwamba County, which will be discussed separately, 3,031 sera were tested in the survey. Only nine of a total of 1,408 children whose sera were included were immune to yellow fever. Of 1,623 specimens from adults, fifty-one from residents of twelve localities were protective.

Although the percentage of the population in Uganda which has been immunized is small, the localities where this has occurred are scattered over a wide area in the central, northern and western sections of the country (Fig 8). It would appear that conditions favourable to extensive outbreaks of the disease in the human population are absent. It is true that large urban centres do not exist and that the population is essentially rural. However, excluding Bwamba, there is no evidence that a rural epidemic, comparable to that which occurred in the Nuba Mountains district of the Anglo-Egyptian Sudan, has ever taken place in Uganda. Nevertheless, limited outbreaks do occur, and the extent of

the area affected suggests that the human population is exposed to infection under conditions which are unfavourable to its spread.

There is ample evidence, in Uganda, of yellow fever virus activity in a non-human host in four localities where studies have been made the incidence of immunity in monkeys greatly exceeds that in man. Furthermore, it has been found that the incidence of immunity in monkeys may rise sharply without

TABLE VIII.  
TANGANYIKA TERRITORY AND ZANZIBAR.

Place.	Year of survey	Number examined.	Number immunized.	Percent age immune.	Age group 6-14 years.			Age group 15 years & over.		
					Number examined.	Number immunized.	Percent age immune.	Number examined.	Number immunized.	Percent age immune.
Tanganyika Territory										
Western Border										
Bugene	41	29	0	0.0	14	0	0.0	15	0	0.0
Kasibo	41	49	0	0.0	16	0	0.0	33	0	0.0
Kakonko	41	70	0	0.0	43	0	0.0	27	0	0.0
Karamba	41	50	0	0.0	26	0	0.0	24	0	0.0
Karoma	41	4	0	—	1	0	—	3	0	—
Kasana	41	27	0	0.0	13	0	0.0	14	0	0.0
Kasanga	41	2	0	—				0	0	—
Kala	41	6	0	—	1	0	—	5	0	—
Kimondo	41	6	0	—	1	0	—	5	0	—
Makara	41	41	0	0.0	23	0	0.0	18	0	0.0
Mugunzu	41	20	0	0.0	10	0	0.0	10	0	0.0
Ngara	41	25	1	2.0	11	1	9.1	14	0	0.0
Nyakanyama	41	30	0	0.0	15	0	0.0	15	0	0.0
Nyashoni	41	30	0	0.0	14	0	0.0	16	0	0.0
Ujiji	41	54	0	0.0	33	0	0.0	21	0	0.0
Total		467	1	0.2	221	1	0.5	246	0	0.0
Zanzibar										
	42	77	0	0.0				77	0	0.0

a parallel rise in humans in the same area. For example studies on Bukoba Island on Lake Victoria, in 1943 showed that the incidence of immunity in monkeys rose from 4 to 89 per cent. during the last 8 months of the year. A survey of the human population on the island in 1944 showed an increase—but only a slight increase—in immunity over that found in previous years (Table IX). The African monkey almost certainly plays a role in the epidemiology of yellow fever in man. It provides a source of virus which, under favourable circumstances, may be introduced into the human population. It cannot, however

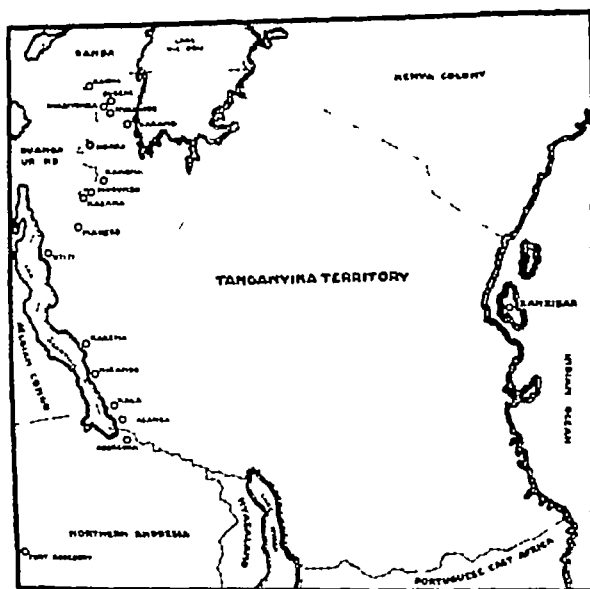


FIG 7 DISTRIBUTION OF IMMUNITY TO YELLOW FEVER IN TANGANYIKA AND ZANZIBAR.

Solid circles denote immunity, blank circles denote no immunity

serve as a true reservoir of the virus, since the individual animal, if it survives the infection as it usually does, rapidly becomes immune to it

Bwamba County has been the scene of our most intensive epidemiological investigations in Uganda since the opening of this Institute because the earliest studies indicated that yellow fever had been more prevalent there than anywhere else in the Protectorate. During the years 1937-40 a total of 2,054 sera were collected in eighty-eight places in Bwamba County. The results of tests with these sera are summarized in Table IX. Since the number of specimens from many places was too small to give significant results the whole area covered by the survey has been divided into thirteen districts as shown in Fig 9. The combined results for all places in each of these districts are given in Table X. Immune adults were found in all thirteen districts, but in only six of them did the findings indicate activity of the virus within the last 15 years. Four of the six districts where immune children were found lie adjacent to, or near, the uninhabited Semliki forest, and the other two adjoin the forested valley of the Lamia river. There is an unequal distribution of immunity in Bwamba, and a careful scrutiny of the survey findings makes it quite apparent that the risk of infection increases as one approaches the border of the Semliki forest. The forested area of Bwamba County may be seen from Fig 9 to be divided into two distinct portions, *viz*, the Semliki and the Ruwenzori forests. The former is the lowland forest in the valley of the Semliki river at an altitude of approximately 2,500 feet. The latter is a mountain forest, the lower timber-line of which varies from 4,000 to 7,000 feet (HADDOW, 1945). It is the

TABLE IX.  
UGANDA.

Place.	Year of survey	Number examined.	Number known.	Percentage known.	Age group 0-14 years.			Age group 15 years & over.			
					Number examined.	Number known.	Percentage known.	Number examined.	Number known.	Percentage known.	
Toro District											
Bwamba	'37	40	2,054	222	10.8	1,144	81	4.8	910	171	18.6
Kanyampere	'37		49	1	2.0				49	1	2.0
West Nile District											
Aranga	'38		73	3	4.1	32	0	0.0	41	3	7.3
Arua	'39		7	0	—				7	0	—
Ayru	'39		88	0	0.0	88	0	0.0	3	0	—
Idu	'39		87	0	0.0	8	0	—	49	0	0.0
Koboko	'38		78	1	1.3	32	0	0.0	44	1	2.3
Okoko	'38		48	0	0.0	4	0	0.0	24	0	0.0
Ora	'39		62	0	0.0	13	0	0.0	49	0	0.0
China District											
Adiak	'37		25	0	0.0	25	0	0.0			
Agoro	'37		47	3	6.4	23	0	0.0	24	3	12.5
Amaga	'37		15	0	0.0	12	0	0.0	3	0	—
Eken	'37		48	0	0.0	20	0	0.0	28	0	0.0
Laguna	'37		8	0	—	8	0	—	1	0	—
Lokung	'37		30	1	3.0	21	0	0.0	29	1	3.4
Madi Ope	'37		48	0	0.0	23	0	0.0	25	0	0.0
Nadung	'37		5	0	—	4	0	—	1	0	—
Padibe	'37		63	0	0.0	27	0	0.0	26	0	0.0
Pajole	'37		30	2	6.7	30	2	6.7			
Palebek	'37		27	0	0.0	21	0	0.0	6	0	—
Bunyoro District											
Kinyala	'39		96	0	0.0	24	0	0.0	72	0	0.0
Kiryadongo	'37		203	7	3.4	128	2	1.6	77	5	6.5
Kwara	'39		45	0	0.0	42	0	0.0			
Kyagwale	'39		66	0	0.0	80	0	0.0	8	0	—
Kigezi District											
Kabale	'40		78	0	0.0	73	0	0.0	5	0	—
Kisoro	'40		48	0	0.0	30	0	0.0	18	0	0.0
Alpalo	'40		67	0	0.0	63	0	0.0	5	0	—
Ankole District											
Bushenyi	'40		62	0	0.0	42	0	0.0	10	0	0.0
Ibanda	'40		61	0	0.0	47	0	0.0	14	0	0.0
Mbarara	'40		63	0	0.0	55	0	0.0	8	0	—

TABLE IX—continued

Place	Year of survey	Number examined	Number immune	Percentage immune	Age group 0-14 years			Age group 15 years & over		
					Number examined	Number immune	Percentage immune	Number examined	Number immune	Percentage immune
Masaka District										
Katera	'41	54	0	0.0	41	0	0.0	13	0	0.0
Masaka & Kalungu	'37	242	5	2.1	34	0	0.0	208	5	2.4
"	'44	58	1	1.7	20	0	0.0	38	1	2.1
Menro District										
Bombo	'37	74	1	1.3	1	0	—	73	1	1.4
Entebbe	'41	50	0	0.0	50	0	0.0			
Mbiru	'43	70	3	4.3				70	3	4.3
"	'44	56	1	1.8	48	1	2.1	8	0	—
Mityana	'41	40	0	0.0	40	0	0.0			
Mpigi	'41	27	0	0.0	27	0	0.0			
Mulono	'37	22	0	0.0				22	0	0.0
Ntenjeru	'41	50	0	0.0	46	0	0.0	4	0	—
Islands—Lake Victoria										
Bunjal o	'42	4	0	—				4	0	—
Buvuma	'42	22	0	0.0	18	0	0.0	4	0	—
Kome	'30-'42	122	6	4.9	77	0	0.0	45	6	13.3
Sese Islands										
Bugala	'38	55	1	1.8				55	1	1.8
"	'43	87	4	4.6	31	1	3.2	56	3	5.4
Bukasa	'38	20	0	0.0	1	0	—	19	0	0.0
"	'43	110	13	11.8	17	0	0.0	93	13	14.0
"	'44	66	7	10.6	16	3	18.8	50	4	8.0
Zinga	'38	5	0	—				5	0	—
Bugishu District										
Bubulu	'41	18	0	0.0				18	0	0.0
Budadiiri	'41	22	0	0.0				22	0	0.0
Bufumbo	'41	20	0	0.0				20	0	0.0
Bulucheke	'41	25	0	0.0				25	0	0.0
Bunkoko	'41	20	0	0.0				20	0	0.0
Bupota	'41	10	0	0.0				10	0	0.0
Busano	'41	10	0	0.0				10	0	0.0
Muyembe	'41	18	0	0.0				18	0	0.0
Simu River	'41	30	0	0.0				30	0	0.0
Sipi	'41	23	0	0.0				23	0	0.0
Teso District										
Amuria	'37	20	0	0.0	4	0	—	22	0	0.0
Total		5,085	282	5.5	2,552	60	2.4	2,533	222	8.8





Semliki forest and its gallery extensions with which the epidemiology of yellow fever in Bwamba is associated. No evidence has, as yet, been found that the Ruwenzori mountain forest is in any way involved.

A description of Bwamba County and of investigations carried out there over a period of years has already been published (MAHAFFY *et al.*, 1942). During the course of these studies yellow fever virus was isolated from a human case in 1941, but the presence of the disease in Bwamba at that time was not suspected until the protection test revealed that a considerable percentage of the population in certain areas had been immunized between January, 1940, and April, 1941. The results of the tests which provided this information are given in Table XI. The donors for this study were residents of four of the districts mentioned above—districts specially chosen because of their proximity to the Semliki forest. Of a group of 171 persons whose sera were retested in 1941, fifty-two, or 30.4 per cent, were found to have developed immunity since October, 1939, or January, 1940, when the original tests were done. It was on the basis of this finding that the study was undertaken which resulted in the isolation of yellow fever virus in Bwamba, later in 1941.

To illustrate further the relationship of forest to virus activity in the human population of Bwamba, Fig. 10 has been prepared to show the individual places where immune persons, both children and adults, have been found. This map shows the places of residence of all the immune donors listed in Tables X and XI. Prominence has been given to localities with immune children,

TABLE X  
BWAMBA COUNTY—UGANDA

Place	Year of survey	Number examined	Number immune	Percentage immune	Age group 0-14 years			Age group 15 years & over		
					Number examined	Number immune	Percentage immune	Number examined	Number immune	Percentage immune
Bubandi	'37-'39	299	20	6.7	134	1	0.7	165	19	11.5
Bubukwanga	'37-'40	79	14	17.7	31	0	0.0	48	14	29.2
Buhundu	'37-'39	58	5	8.6	31	0	0.0	27	5	18.5
Bukangama	'37-'39	20	2	6.9	13	0	0.0	16	2	12.5
Bundibugyo	'37-'39	382	53	13.9	195	7	3.6	187	46	24.6
Bundingoma	'37-'40	134	15	11.2	76	5	6.6	58	10	17.2
Bundinjongo	'37-'39	68	2	3.0	31	0	0.0	37	2	5.4
Bunyangule	'37-'39	84	4	3.6	48	0	0.0	36	4	11.1
Busaro	'37-'39	274	27	9.9	153	0	0.0	121	27	22.3
Hakitara	'37-'40	303	38	12.5	218	18	8.2	85	20	23.5
Hakutengya	'37-'40	210	36	17.1	140	19	13.6	70	17	24.3
Kirmia	'37-'39	95	2	2.1	64	0	0.0	31	2	6.5
Ntotoro	'37-'39	39	4	10.3	10	1	10.0	29	3	10.3
Total		2,054	222	10.8	1,144	51	4.5	910	171	18.8

TABLE VI.

RESULTS OF TESTS IN 1941 WITH SERA OF BWAMBA RESIDENTS KNOWN TO BE NON-IMMUNE IN 1939 OR 1940

Area.	Number examined.	Number immune.	Percent age immune.	Age group 0-14 years.			Age group 15 years & over.		
				Number examined.	Number immune.	Percent age immune.	Number examined.	Number immune.	Percent age immune.
Babukwanga	3	2	—	4	1	—	1	1	—
Bondrogona ...	14	6	42.9	10	5	50.0	4	1	—
Hakikara ...	23	23	100.0	62	46	74.2	21	7	33.3
Kakibungya ...	69	11	15.9	57	7	12.3	12	4	33.3
Total	171	42	24.6	133	29	21.8	38	13	34.2

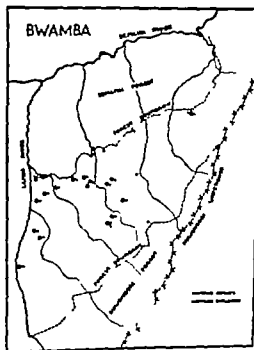


FIG. 10. LOCALITIES WHERE IMMUNE CHILDREN AND NON-IMMUNE ADULTS WERE FOUND IN BWAMBA COUNTY UGANDA.

Large circles denote immunity in children; small circles denote immunity in adults.

since children are much more likely to have been immunized in the immediate vicinity of their present homes than is the case with adults. Although adult immunity occurs throughout the country the percentage is higher in the population living in close contact with the Semliki forest. No immune children were found in the grassland areas which lie south of this forest and at some distance from it.

The results of long-continued studies in Bwamba County have led us to the conclusion that we have in that area an endemic focus of yellow fever. Following the isolation of virus in 1941, a mass vaccination campaign was carried out there and subsequent studies have shown that 93 per cent of the population were immune 3 years later. Despite this fact yellow fever virus has, on two occasions since 1941, been isolated from mosquitoes caught in or near the Semliki forest (details to be published later). We therefore have reason to believe that the virus of the disease is able to maintain itself in Bwamba for an indefinite period and that its continued presence is not dependent on the existence of a susceptible human population. Our knowledge of the factors responsible for the persistence of the virus is by no means complete and intensive epidemiological studies are being continued in the area.

#### DISCUSSION

The results of the yellow fever protection test survey in Central and East Africa have confirmed earlier findings and have added to our knowledge of the past incidence of the disease in that part of the Continent. Recently infected areas in which yellow fever was not previously known to have existed include Eritrea, western Somalia, central Kenya and the Balovale district of Northern Rhodesia. The fact that the disease is now known to have occurred as far east as the Red Sea coast of Eritrea and as far south as Balovale does not necessarily indicate a recent invasion of these places. It may indicate only an extension of our knowledge of the distribution of the infection.

That clinical cases of yellow fever have never been recognized in any of the territories mentioned above is neither disturbing nor surprising to anyone who is familiar with the recent history of the disease in Africa. The protection test provided the first indication that the disease had occurred in the Anglo-Egyptian Sudan. Recognition of cases came later when the most extensive epidemic ever recorded in any part of Africa was observed in the Nuba Mountains (KIRK, 1941). The difficulty in finding and diagnosing yellow fever in the African is well illustrated by our own experience in Bwamba County in Uganda. Here again the protection test indicated that the disease had been present within recent years but the most careful search by experienced workers failed to reveal a single case during a 4-year study. It was only after the protection test demonstrated the existence of the disease, in a restricted area, within *recent months*, that our efforts were successful. The explanation for this lies, in part at least, in the fact that a high percentage of cases in Africans are mild, and

mild yellow fever cannot be diagnosed clinically. These are points which are still by no means universally appreciated.

Severe and fatal cases of yellow fever presenting the classical symptoms of the disease, do occur in Africa, but they are so infrequent that, under the conditions which prevail there, the fact that they have not been seen and recognized does not constitute a sound basis on which to declare an area free of the infection. Consequently until such time as methods, such as a viscerotomy service, which will give us more up-to-the minute information on the incidence of the disease, can be developed in Africa, we must be content with the knowledge provided by the immunity survey. On the assumption that where yellow fever has occurred it can and may occur again, we are justified in regarding the whole area within which immunity has been demonstrated as constituting the endemic zone in Africa. In Fig. 1 (page 59) we have shown the approximate boundary of this zone as far as it is known at the present time.

### SUMMARY

1 The results of a yellow fever protection test survey covering the examination of 10,274 sera collected in ten countries in Central and East Africa are presented.

2 The findings have demonstrated that yellow fever has occurred recently in the Belgian Congo Uganda, the Anglo-Egyptian Sudan, Eritrea, Somalia, Kenya and Northern Rhodesia. Abyssinia has not been adequately studied and Tanganyika Territory and Zanzibar have been free of recent infection.

3 The disease has occurred within recent years as far east as the Red Sea coast of Eritrea and as far south as Balovale in Northern Rhodesia.

4 The area within which immunity has been demonstrated should be regarded as the endemic zone of yellow fever in Africa. The approximate boundary of this zone has been indicated.

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## THE INCIDENCE OF SICKLAEMIA IN WEST AFRICA

BY

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Since HERRICK (1910) first described an anaemia associated with sickling of the red cells in a native of the West Indies, the sickling phenomenon has aroused interest in America where it has been recognized as an important factor in the pathology of the negro

Under the term sicklaemia (COOLEY and LEE, 1926) are included those cases of anaemia with periodic haemolytic crises and those, more numerous, which without clinical symptoms exhibit sickling of the red cells *in vitro*, whenever the oxygen tension is reduced—the sickling trait

In West Africa, the ancestral home of most American negroes, sicklaemia has received scant attention apart from record of individual cases by RUSSELL and TAYLOR (1932), SMITH (1934) and REID (1936), and the fuller and more recent studies of EVANS (1944 and 1945) The present communication deals with the incidence of sicklaemia in the British West African Colonies

### INCIDENCE OF SICKLING

As the majority of investigations were made under field conditions, the technique employed was simple After cleaning the skin, a drop of blood was

\* Our thanks are due to Brigadier J B A WIGMORE, D D M.S., West African Command, for permission to publish these results Dr A H CHENNARD and Dr W J MCCLINTOCK, of the Colonial Medical Service, very kindly placed patients at our disposal, while Miss D BARNFULL and Mr A Y KPEGLO gave us much help and assistance

placed on a slide covered with a clean cover glass and ringed with vaseline. Ringed preparations, which must be protected from the depredations of ants, were kept at room temperature and examined after 24 hours.

In all 5,500 Africans were examined of whom 12.4 per cent. showed the sickling trait. Just over half the total number were natives of the Gold Coast and French Togo the remainder came from Gambia, Sierra Leone and Nigeria, where southern tribes, pagans from the Plateau and Hausas were examined. Sickling was found in all tribes and no significant specific tribal differences were noted.

The sex incidence of sickling was approximately equal, 11.2 per cent. in males, 12.6 per cent. in females. Differences were noted in relation to age (see table). Although the numbers from the two extremes of life were small they suggest that the expectation of life in sicklers may be less than in normal persons.

The incidence of sicklaemia in relation to certain physiological and pathological conditions was investigated.

### 1. *Pregnancy*

REID (1936) pointed out that a sickling crisis in the later months of pregnancy was often fatal.

Of 455 pregnant women examined in Accra, seventy seven, or 16.9 per cent., sickled. REID (1944) found thirteen sicklers among 100 pregnant women in the Gold Coast.

### 2. *Sterility*

Of sixty-one women who either had never conceived or had failed to produce a viable foetus, thirteen, or 21.3 per cent. sickled.

### 3. *Lunacy*

Of seventy-six lunatics of both sexes, aged 35 to 60 years, five, or 6.5 per cent. sickled. This rate is comparable with that in old people without mental disease.

### 4. *General Morbidity*

Among 296 hospital patients not suffering from mental disease, pneumonia or ulcers, forty-seven, or 15.8 per cent., sickled.

### 5. *Pneumonia*

The incidence of sickling in Africans with pneumonia was investigated to determine if the jaundice seen in negroes with pneumonia can be correlated with sicklaemia. In South Africa, GELFAND and LEWIS (1942) found jaundice in 10 per cent. of negroes with pneumonia. Among 1,240 consecutive cases of

pneumonia in West African soldiers, sixty-five, or 5.2 per cent, had jaundice. Of these 1,240 pneumonia patients, 125 sickled, and of the 125 sicklers twelve (9.6 per cent) had jaundice. Among the 1,115 non-sickling pneumonia patients, fifty-three, or 4.7 per cent, had jaundice. The difference in the incidence of jaundice in sicklers and non-sicklers is not statistically significant.

### 6 Ulcers

The association between sickle cell anaemia and leg ulcers was described by HERRICK (1910). Later observers have estimated that in America ulcers of the leg occur at one time or another in the history of 40 per cent of negroes with sickle cell anaemia. DIGGS *et al* (1933) could not determine any correlation between the sickling trait and the presence among American negroes of active ulceration or old scars on the legs. In West Africa, of 103 patients with tropical ulcers of the leg, twelve, or 11.6 per cent, exhibited sicklaemia.

### SICKLING IN RACES OTHER THAN THE NEGRO

From time to time sicklaemia has been described in whites in whom, so it is alleged, there is no negro ancestry. All cases have come from the Mediterranean area or America. In the former, since Roman times there has been a large infiltration of negroes, while in America negro ancestry may be vehemently denied but is not easily excluded. DIGGS *et al* (1933) examined 309 whites from Memphis, SYDENSTRICKER (1924), 1,000 from the Southern States, and KORB and MIYAMOTO (1927), 100 from St. Louis, all were negative.

Five hundred and sixty-eight British soldiers and airmen, temporarily stationed in West Africa, were all negative, 188 Syrians were also negative.

TABLE.

INCIDENCE OF SICKLING IN RELATION TO AGE (BOTH SEXES) IN NORMAL AFRICANS

Group	Number examined	Number sickling	Percentage with sickling
New-born babies (cord blood)	25	9	36.0
Infants (1 to 5 years)	243	29	11.9
School children (6 to 15 years)	455	44	9.6
Students (16 to 20 years)	426	43	10.9
Adults (21 to 50 years)	1,510	182	12.7
Old people (60 years and over)	50	2	4.0

### DISCUSSION AND CONCLUSION

Among 5,500 West Africans examined, 682, or 12.4 per cent., had sicklaemia. In the United States of America the results of seventeen investigators,



summarized by LEWIS (1942), showed that among 11 121 negroes examined, 832 (7·48 per cent.) sickled. Variations were from 4·3 per cent. in Georgia to 15 per cent. in North Carolina. TOMLINSON (1945) among 3,000 examinations in the Panama Canal zone, observed 11·2 per cent. of sicklers. In West Africa, EVANS (1944) found that among 561 African soldiers 112 (19·9 per cent.) sickled. In Gambians the rate was 28·3 per cent.

#### Conclusions

Among 5,500 West Africans examined, the incidence of sicklaemia was 12·4 per cent.

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## COOLEY'S ANAEMIA NOTES ON SIX ADULT CASES

BY

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Recent work in America on families of Greek and Italian origin by SMITH (1943) and DAMASHEK (1943) has left no doubt that the fatal familial blood-dyscrasia first described by COOLEY (1927) is the most serious form of a disease of varying severity transmitted as a Mendelian dominant by less-severely affected "carriers". There is, indeed, a continuous gradation of the degree of blood-disorder—from a fatal anaemia with gross physical change (splenomegaly, hepatomegaly, cardiac enlargement, universal bone-changes, icterus) and obvious dys-haemopoiesis, through a moderately severe and disabling refractory hypochromic anaemia with slight physical changes (palpable spleen, slight thickening of the calvarium of the skull) and well-marked morphological anomalies of the erythrocytes but absence of circulating normoblasts—to the mildest disorder apparent only on careful laboratory examination of the blood in a perfectly healthy individual.

The haematological features which constitute the "trait" found in healthy relatives of the anaemic persons are a degree of anisocytosis and poikilocytosis out of proportion to the intensity of the anaemia—indeed, sometimes accompanying a hypochromic erythrocytosis—the presence of target, oval, and stippled cells (usually), and increased resistance to haemolysis by hypotonic

\* My thanks are due to the DIRECTOR OF MEDICAL SERVICES, Cyprus, for permission to publish this paper and to the doctors who referred cases to me, also to Mr N SCHIZAS, who assisted me technically.

saline. The sternal marrow shows an increase in the proportion of normoblasts to other nucleated cells (WINTROBE *et al.*, 1940). It is not yet possible to say which characteristics of the red cells are absolutely pathognomonic of the trait but the fundamental defect is probably the production of an abnormally thin cell with a deficient haemoglobin content, and the laboratory test which most strikingly shows the presence of the disorder is the plotting of the fragility curve.

The harbouring of the trait is of no consequence to the individual, though it may be to his offspring so he usually survives unrecognized to adult age. Little notice has yet been taken, in Cyprus at least, of the clinical features of the intermediate group who reach and pass puberty but yet may be seriously incapacitated and as the disease may apparently spring up in diverse corners of the world it has seemed worth while to record the findings in these Cypriots. A description of the severe disease as seen in childhood in Cyprus was given in a previous paper (1944).

#### CLINICAL FINDINGS.

**Case 1** N. S., male, age 22. No history of severe anaemia in the family. Recurrent fever (? malaria) in childhood but little restriction of activity. Moderate normal physique, slight icterus. Spleen I (Hackett). No other abnormal signs. No thickening of calvarium. Haemoglobin 80 per cent. R.C.B., 5.7 millions per c.mm.

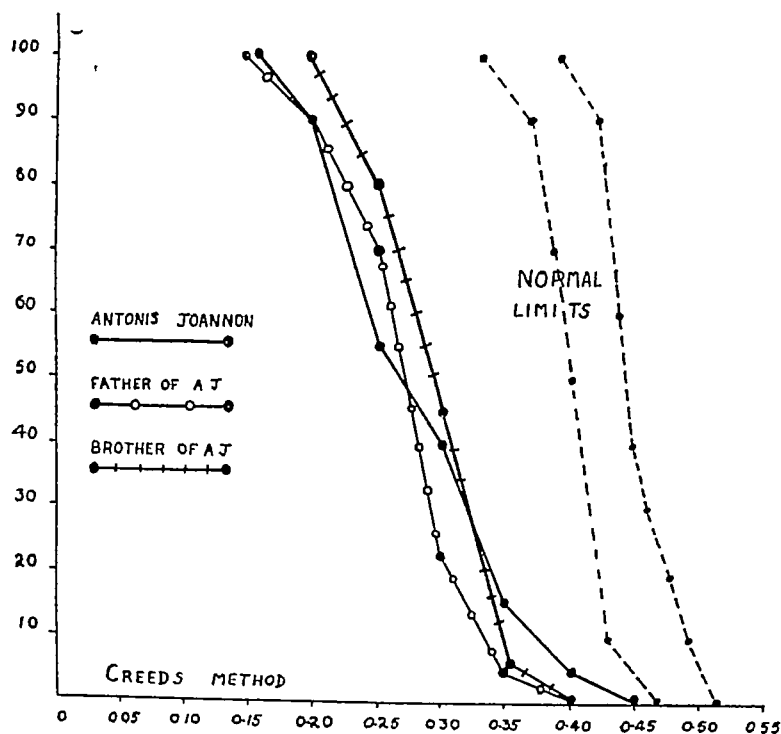
**Case 2.** L. L., male, aged 30. No family history of anaemia. Fever (? malaria) at 15. Normal childhood activity. He has been worried about the size of his spleen and his yellow complexion since the age of 20. Married, with one normal child (not examined).

Normal adult physique. Slightly mongoloid facies. Marked icterus of conjunctivae. Liver 2 f.b. below costal margin. Moderate degree of thickening of calvarium.

At 28 a splenectomy was carried out and he states that the icterus diminished, and he improved subjectively. Records of his blood-count show that both before and after this he had usually between 5 and 5.5 million red cells per c.mm. His haemoglobin showed a dubious rise from 70-80 per cent. to 90 per cent. But there is no doubt that a typical normoblastic response took place, such as is described in children with Cooley's anaemia by BARR *et al.* (1932). The same technician recorded some circulating normoblasts prior to the operation and subsequently numerous, the latter referring to counts of 10 and 36 thousand per c.mm. at 1 and 29 months respectively after wards. When seen by me 2½ years later he still had 23 thousand normoblasts per c.mm. Haemoglobin 90 per cent. R.B.C., 5.9 millions per c.mm.

Sahli, 100 per cent. = 14.56 grammes Hb. per 100 c.c.

Case 3 A J, male, aged 20 An uncle was known to have been very pale, and died at 24 His own development was normal till 4 years old, increasing pallor and splenic enlargement followed a febrile illness at that date, he could never take strenuous exercise when at school Genital development complete but rather late even now he only shaves once every 2 to 3 days



Fragility curve of Case 3, and of his physically normal brother and father, compared with normal (English) range

Normal height but a large body with thin limbs Firm bony union at the site of an old fracture of radius Icterus of conjunctivae and mongoloid facies Moderate enlargement of axillary lymph nodes Slight cardiac enlargement with soft systolic murmur Abdomen not visibly distended, but liver enlarged 2 f b below costal margin, spleen III, hard and smooth Calvarium slightly thickened Haemoglobin, 35 per cent R B C, 2.8 millions per c mm

Case 4 M I, female, aged 27 Her brother's child died at 17 after a splenectomy for splenomegaly and pallor She was well till 5 years old Then several years of intermittent fever with enlargement of the spleen As a child she was too weak to play with the other children or go to school and her feet swelled after exertion Menses seen on only four or five occasions several years ago, since when there has been complete amenorrhoea Recently she was

treated for some time as congenitally syphilitic on the strength of a 1+ W.R. and the flattened bridge of her nose the latter on questioning, was found to date from a fall from a donkey when a small child. There were no genuine signs of syphilis.

Poorly developed musculature of limbs slightly mongoloid faces scarred depressed bridge of nose Scanty axillary and pubic hair Pitting oedema of ankles. Systolic apical murmur Abdomen distended by enlarged spleen (II), enlarged liver and free fluid. Slight thickening of the calvarium the tibia showed osteoporosis with prominence of the trabeculae. Haemoglobin, 35 per cent. R.B.C. 3.3 millions per c.mm.

Case 5 M. M. J. male, aged 20 His sister suffered from the same complaint and died at 18. The disease appeared when he was 2 years old with fever and progressive weakness. He attended school but was never able to take strenuous exercise Persistent attention from many doctors has left him disillusioned and apathetic he refused sternal puncture and objected strongly to venepuncture. Semi-invalid existence

General physique poor (height, 1.58 m. weight, 43 kg.) and his limbs were small and thin in comparison with the trunk. Mongoloid appearance slight but features quite different from parents. Conjunctivae icteric. Genital development apparently normal but voice still prepubertal. Soft systolic murmur over praecordium. Abdominal distension not marked in spite of enlarged spleen (II) and liver (3 f.b. below costal margin). No recognizable thickening of calvarium. Haemoglobin, 35 per cent. R.B.C. 3.4 millions per c.mm.

Case 6 M. Ch. aged 20 male. A full description of this patient was given in the previous paper on Cooley's anaemia in Cyprus (FAWDEY 1944).

Onset at 1½ years. All the changes recorded in the childhood disease greatly exaggerated Stunted physical development, infantilism, exophthalmos and gross thickening of the calvarium. Haemoglobin 25 per cent. R.B.C. 2.8 millions per c.mm.

#### COMMENTARY

All six patients showed splenomegaly hypochromic erythrocytes, and increased resistance of the cells to haemolysis by hypotonic saline.

Case 5 showed a great increase of normoblasts in the marrow

Cases 1 and 2 are typical of the mildest degree of the affliction the first had no symptoms directly referable to his blood-dyscrasia and the second only the icterus of his conjunctivae and the possession of a spleen large enough to attract a surgeon. Having no anaemia, there has been no interference with their physical capabilities. Cases 3, 4 and 5 all lead a somewhat miserable existence, the first two unable in their villages to earn their living with their hands (they were each given a trial as domestic servants by the writer) and the third, though

TABLE  
RELEVANT HAEMATOLOGICAL FINDINGS

Case number	1	2	3	4	5	6
Red cells—						
Anisocytosis	+	+	++++	++++	++++	++
Poikilocytosis	+	+	++	++	+	++
Target cells	+	+	+	+	0	+
Circulating normoblasts	+	++++	+	+	+	++
*Fragility, % Saline at which haemolysis started, reached 50%, was complete	40 25 15	40 25 15	45 25 15	45 25 10	40 20 10	45 25 10
Marrow Percentage of normoblasts	45	60	61	63	—	76

\* Creed's method using oxygenated venous oxalated blood

well educated and living with his parents in a town, too despairing to try to overcome his handicap Case 6, though physically the most affected, nevertheless with a cheerful and determined spirit, leads a fairly active life assisting in his father's business

Except possibly for lifelong repeated transfusion, which is scarcely practicable in Cyprus, there is as yet no therapy whereby the anaemic patients can be restored to health and usefulness in the community

Since these notes were made, other cases have been seen whose degree of anaemia is intermediate between that of Cases 2 and 3, one was a woman of about 35, the other a girl of 16 No doubt the milder form of the disorder will be found to be of fairly frequent occurrence throughout the island It is regretted that time was not available for a fuller enquiry into the histories and haematology of the relatives

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## PENICILLIN IN THE TREATMENT OF EXPERIMENTAL RELAPSING FEVER IN RATS

BY

E S ANDERSON, MAJOR, R A M C \*

*Pathologist*

The experiments described below were carried out on white rats inoculated with the Cyprus strain of *Borrelia recurrentis*. The human disease caused by this organism is a tick-borne infection (*Ornithodoros tholozani*), and is apparently insensitive to arsenicals.

It was felt that the uncontrolled treatment of human cases could present little reliable information, since the number of such cases is small, and the disease is notoriously irregular in its behaviour, even when untreated. Thus, the number of relapses varies from one to four or more, and some of the later relapses are so fleeting in character that the spirochaete may easily be missed if prompt examination of the blood is not carried out. Also, the interval between the initial pyrexia and the first clinical relapse has been, on occasion, as long as 24 days.

Although there is little uniformity in the incidence of the organism in the blood of infected untreated animals, the white rat offered the best possibility of providing useful data, because of the relative ease of infecting large numbers of these animals simultaneously.

The protracted persistence of the spirochaete in the central nervous system of the rat, and its easy demonstration by the inoculation of emulsified cerebral substance into further rats, are of great assistance in disproving apparent cures with penicillin.

### OUTLINE OF EXPERIMENT

An attempt was made to cover most angles of treatment, and the experiments were therefore divided into three groups.

\* I am greatly indebted to Colonel H T FINDLAY, D D P, M E, for his encouragement and many valuable suggestions in the preparation of this paper, and to Prof S ADLER, of the Department of Parasitology, the Hebrew University, Jerusalem, for permission to quote his unpublished results of an investigation of a type similar to that described.

The present paper was submitted for publication early in 1945.



Group I.—The prophylactic administration of penicillin simultaneously with, or within a very short time of the inoculation of infected material.

Group II.—The treatment of the developed disease.

Group III.—Observation of the behaviour of the spirochaete when mixed with a solution of penicillin *in vitro*.

Further subdivision was effected from the point of view of dosage. It seemed possible that a massive single dose might succeed in ridding the animal of spirochaetes where intermittent smaller doses might fail, particularly when the drug was to be employed prophylactically. Group I was therefore divided into —

(a) Intermittent dosage commencing 3 hours after inoculation.

(b) Single massive dose at time of inoculation no subsequent treatment.

(c) Massive dose at time of inoculation, followed by a similar dose 3 hours later

(d) Single massive dose at time of inoculation, followed by intermittent (3-hourly) dosage commencing after a further 3 hours.

Group II was divided as follows —

(a) Intermittent dosage commencing from the time the animal first yielded a positive blood.

(b) A very heavy single dose in an animal known to be positive in spite of prophylactic treatment. For this experiment an animal from Group I (c) was utilized.

Young white rats were used of a strain proved to be susceptible as a general rule, to *Borrelia recurrentis*. They comprised two litters of the same age, and averaged 85 grammes in weight—*i.e.* about 1/1,000 of the average human body weight. It was therefore apparent that in order to achieve a concentration of penicillin similar to that obtained in the human subject, 1/1,000 of the average human dosage should be utilized. Since 15 000 units is the usual human intermittent dose 15 units would have constituted a comparable concentration in the rats, but in order to give failures in treatment a greater significance it was decided to utilize ten times this dosage, *i.e.*, an intermittent dose of 150 units 3 hourly.

The single massive dose was calculated on exactly the same principle and, when administered, was equivalent to 24 hours dosage at once, that is 1,200 units. In order to give a rough estimate of rat treatment in equivalent terms of human treatment, the dose is multiplied by 1,000, *g.*, 150 units rat = 150 000 units human. 1,200 units rat = 1,200,000 units human and so on.

The drug was injected intramuscularly into the thigh in every case.

All penicillin employed was assayed by Rammelkamp's technique and showed at least the potency claimed by the manufacturers.

Six animals of the same litters were inoculated with infected material and used as controls.

### Technique of Infection

An adult rat was infected either with blood from a human case, or with a saline emulsion of the brain of a rat infected previously. In either case the intraperitoneal route was utilized, and 5 c c of infected material was introduced. When the animal developed a heavily positive blood (a minimum of 10 spirochaetes to the 1/12-inch field in a thick film) 5 to 7.5 c c. of blood were drawn off by cardiac puncture. A 1½-inch No 19 gauge needle with a short bevel and specially sharpened point, was used for this operation, which is rarely fatal.

The blood was defibrinated, aspirated into a second sterile syringe, and injected intraperitoneally into the experimental animals, in equally divided doses. If insufficient blood was available, it was diluted with sterile normal saline until the desired volume was reached. A minimum of 0.1 c c of undiluted blood was introduced into each animal.

The animals were examined at least once every 24 hours following inoculation, by making thick films from the clipped tail.

### RESULTS

#### GROUP I PROPHYLACTICALLY TREATED ANIMALS

(a) Intermittent dosage commencing 3 hours after inoculation, and maintained 3-hourly for 78 hours, 26 doses, total, 3,900 units

TABLE I

RATS 1 AND 2 TREATED WITH PENICILLIN 150 UNITS 3-HOURLY COMMENCING 3 HOURS AFTER INOCULATION AND MAINTAINED FOR 78 HOURS  
Neither of the animals showed a positive blood at any time

Day following inoculation	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Rat 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rat 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

#### Key to Tables

++++ Parasites uncountable      + Less than 10 parasites to 1/12" field  
+++ 50-100 parasites to 1/12" field      — No parasites found in 100 1/12" fields  
++ 10-50 parasites to 1/12" field

(b) Single dose of 1,200 units at time of inoculation. No subsequent treatment

TABLE II

RATS 3 AND 4 TREATED WITH PENICILLIN 1,200 UNITS AT TIME OF INFECTION. NO FURTHER TREATMENT

Day following inoculation	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Rat 3	—	—	—	+	+	+	—	—	—	—	+	+	—	—	—
Rat 4	—	—	+	+	—	—	+	—	—	—	—	—	—	—	—

(c) 1,200 units at time of inoculation followed by —

(1) A further 1,200 units after 3 hours. This animal (Rat 5) showed spirochaetes on the 2nd day and relapsed on the 4th day. It was also employed in a later experiment, Group II (8).

(2) Intermittent dosage 3 hourly for 78 hours—an additional 3,900 units. No evidence of infection developed in this animal, Rat 6.

TABLE III.

RATS 5 AND 6 TREATED WITH PENICILLIN 1,200 UNITS. TIME OF INOCULATION  
 RAT 5.—FURTHER 1,200 UNITS AFTER 3 HOURS, 10,000 UNITS 6TH DA  
 RAT 6.—100 UNITS 3-HOURLY COMMENCING AFTER 3 HOURS, MAINTAINED FOR 78 HOURS.

Day following inoculation.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Rat 5	...	+	-	+	+	10,000 units ++	-	-	-	-	-	-	-	-	-
Rat 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(d) 1,200 units 3 hours after inoculation. No further treatment. Both animals, Rat 7 and Rat 8 were positive on the 2nd day and both relapsed later.

TABLE IV

RATS 7 AND 8 TREATED WITH PENICILLIN 1,200 UNITS 3 HOURS AFTER INOCULATION.  
 NO FURTHER TREATMENT

Day following inoculation.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Rat 7	+	-	-	-	-	+	-	+++	+	-	-	-	-	+	-
Rat 8	+++	+	+	++	+	-	-	-	+	-	-	++	-	-	-

#### GROUP II. TREATMENT OF THE DEVELOPED DISEASE.

(a) Intermittent dosage commencing after the first positive blood film 150 units 3-hourly maintained for 54 hours. Total, 2,700 units. No spirochaetes were found on the day following the commencement of treatment. The 8th day brought a momentary relapse and the 12th day a more substantial one. (Table V.)

TABLE V

RAT 9 TREATED WITH PENICILLIN 150 UNITS 7 HOURS COMMENCING FROM TIME RAT  
YIELDED POSITIVE BLOOD TREATMENT MAINTAINED FOR 54 HOURS

Day following inoculation	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Rat 9	+++	-	-	-	-	-	+	-	-	-	+	+++	++++	-	-

(b) Rat 5, used in Group I (c), was given 10,000 units on the 6th day, at which time the blood contained numerous spirochaetes. The blood was negative on the 7th day, and did not show further spirochaetes during the period of observation. On the 29th day following infection, the animal was sacrificed, the brain emulsified in normal saline, and introduced into the peritoneal cavity of a fresh rat. The latter showed a positive blood 2 days later.

#### GROUP III *In vitro* EFFECTS OF PENICILLIN ON *Borrelia recurrentis*

Heavily infected blood was mixed with penicillin in normal saline, 1,000 units per c.c., in the proportion of 1 blood/2 penicillin. A control of infected blood diluted with normal saline in the same ratio was also prepared, and the behaviour of the organisms compared by simultaneous observation under two microscopes, for more than an hour, at 26° to 30° C. No difference in behaviour was noted.

Further examination with the dark field microscope yielded a like result. Morphologically, and in motility, the spirochaetes in penicillin behaved exactly similarly to those in normal saline.

The suspensions were placed in the refrigerator overnight (10 hours) and subsequently observed microscopically for a further hour at 26° C. No

TABLE VI

RAT 10 TREATED WITH PENICILLIN MIXED WITH HEAVILY INFECTED BLOOD IN PROPORTION  
1 BLOOD/2 PENICILLIN (1,000 UNITS PER C.C.)  
1 HOUR AT ROOM TEMPERATURE (26° C.)  
10 HOURS IN REFRIGERATOR.  
1 HOUR AT 26° C.

Day following inoculation	1		2		3		4		5	6	7	8	9
	M	E	M	E	M	E	M	E					
Rat 10	-	-	-	+	+	+	+	+	++	+	+	+	+

apparent morphological change had occurred in the organisms suspended in penicillin and again they behaved in precisely the same manner as those in saline. Finally 0.15 c.c. of the blood-penicillin mixture was injected intraperitoneally into Rat 10. This became positive 2 days later. It had received the equivalent of 0.05 c.c. of infected blood the spirochaetes in which had been exposed for more than 12 hours to a concentration of penicillin far exceeding that ever attained in treatment.

#### CONTROLS

Six animals were used. Rats 11 to 16

The results demonstrate the considerable variation in the incidence of the organism in the blood during the initial attack and in the relapses. One animal (Rat 13) failed to show a positive blood. Another (Rat 15) became positive only on the 6th day and never subsequently.

TABLE VII.

Day following inoculation.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control rat 11	+++	-	-	-	-	+	+	+++	+	-	-	+	+	-	-
12	+++	-	-	-	+	++	++	-	-	-	+	++	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
15	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
16	-	-	-	+	+	+++	+++	-	-	-	-	-	-	-	-

#### DISCUSSION

It must be noted that the total number of animals employed in the prophylactic and therapeutic series was too small to raise the results to a statistical level of significance. Generalization from these experiments is therefore impossible, although, as will be seen, the major weight of evidence does not favour penicillin as an effective therapeutic agent.

Reviewing the experiments in prophylaxis, it is apparent that those rats in which dosage was intermittent (Rats 1, 2 and 6) did not show signs of infection. This argues a possible spirochaetocidal effect of penicillin. It must be borne in mind, however, that on occasion the infection may be very light so that even prolonged search fails to discover the organism (Table VII).

There was, unfortunately, a shortage of animals at the time, and a repetition of the intermittent treatment series was impossible.

Certain results are quite clear cut, and require little further discussion. These are found in Rats 3, 4, 5, 7 and 8, all of which had varying types of massive prophylactic dosage with penicillin. Every one of these animals developed the infection, and all relapsed. This indicates that heavy doses of penicillin over a limited period are without effect on this strain of *Borrelia recurrentis* once it has gained access to the body. A similar result was obtained by LOURIE and COLLIER (1943) working with *B. duttoni*.

Turning now to the treatment of the disease once it has developed, the ineffectiveness of the drug is again apparent. With intermittent dosage (Rat 9), the organism had disappeared from the blood within 24 hours of the commencement of treatment. This is without significance, however, since a similar disappearance often occurs within a very few hours in untreated animals. The factor of importance is the reappearance of the spirochaete on the 8th and 12th days, indicating that the attempted sterilization had failed.

In the case of massive therapeutic dosage, Rat 5—an animal which had developed the infection in spite of prophylactic treatment—was given the relatively heroic dose of 10,000 units on the 6th day. The apparent cure, as shown by the absence of spirochaetes in the blood from then on, was negated by the demonstration of the infectivity of the cerebral substance. From this it is possible to conclude, either that penicillin even in high concentration was without effect on the spirochaete, or that it may have been effective in the blood but was unable to reach those organisms which seem to become tissue spirochaetes of the central nervous system at an early stage of the disease. Of the two, the former view seems more acceptable.

The *in vitro* experiment demonstrates quite clearly that the drug had no effect on the viability and reproductive power of the organism. Since the work of BIGGER (1944), it has been repeatedly shown that penicillin has a bactericidal effect on susceptible organisms. It might reasonably be expected that, even if the action of penicillin on the spirochaete were not lethal, an experiment of the type described would at least render the organism susceptible to the *in vivo* protective processes, if it were in any way sensitive to the drug. The development of infection in Rat 10 shows that the *in vitro* effect was negligible.

Some workers (LOURIE and COLLIER, 1943, HEILMAN and HERRALL, 1943, AUGUSTINE, WEINMAN and McALLISTER, 1944) report success in the treatment of experimental relapsing fever with penicillin. LOURIE and COLLIER used *B. duttoni* in mice, and HEILMAN and HERRALL used *B. novyi* in mice. Both sets of workers demonstrated the efficacy of penicillin treatment by the increased survival rate and disappearance of the spirochaete from the blood of infected animals. Final proof of elimination of the infection by inoculation of emulsified cerebral tissue from treated mice into susceptible animals does not seem to have been attempted.

Prof S ADLER and Dr R. ASHBEI, using strains of *Borrelia* similar to those of the writer have carried out a comparable series of experiments, also with negative results (personal communication).

It is well known that some tick-borne strains of relapsing fever are resistant to arsenicals. This is particularly true of the Cyprian infection, and a case was encountered in which a fifth relapse occurred in spite of arsenical treatment. Such strains may possess a parallel resistance to penicillin, which would explain the divergent results.

#### SUMMARY

1 Massive doses of penicillin administered over a short period were ineffective in the prophylactic treatment of *Borrelia recurrentis* infection in the white rat.

2 Intermittent prophylactic dosage appeared to prevent the development of the disease.

3 Intermittent dosage did not control the developed disease.

4 Persistence of the infection in the central nervous system of a rat was uninfluenced by relatively huge doses of penicillin administered early in the disease.

5 *In vitro* exposure of the spirochaetes to the action of high concentrations of penicillin did not affect their structure, motility or infectivity.

6 The above conclusions may only be applicable to certain strains of *Borrelia*, since other workers report success in the treatment of experimental relapsing fever with penicillin.

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## COLLAPSE OF THE LUNG AND POROCEPHALOSIS

BY

FRANCIS E STOCK, MB, BS, FRCS, DTMH,  
*Formerly Medical Officer, Colonial Medical Service, Nigeria*

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Infestation with the larva of *Armullifer armillatus* (*Porocephalus armillatus*) is rarely diagnosed during life for only rarely does it produce symptoms. In an excellent review of the literature, CANNON (1942) states that "almost all the infestations of man recorded have been discovered accidentally at autopsy when the parasites appear to have no connection with the fatal termination". He records from the literature, however, fourteen cases and adds a fifteenth case himself, in which at autopsy infestation with *A. armillatus* was found to be the cause or a contributory cause of death. In these fifteen cases the frequency with which the various organs were affected was as follows: Lungs and pleurae, 6; liver, 5; meninges, 2; lymphatic glands, 1; intestines, 1. A few cases have also been recorded in which *Armullifer* infestation has been demonstrated during life radiographically or during surgical operations. No case has so far been recorded in which pathogenic infestation with *A. armillatus* has been diagnosed during life.

The case of lobar collapse of the lung recorded here cannot with certainty be said to be due to infestation with *A. armillatus*, but the apparent absence of any other cause, together with the presence of a calcified nymph in the immediate vicinity of the bronchus, is very suggestive. Writing of cysticercosis, MANSON-BAHR (1940) states that "after a variable period determined partly by the resistance of the host, the parasites die and often undergo a calcareous change" and "the tissues surrounding dead and degenerating cysticerci undergo active degenerative changes with marked cellular response". The first statement certainly applies in equal measure to porocephalosis, and there is no reason to suppose that the second does not also apply. Whether this reaction is due to the dead parasite itself or to the sudden release of fluid from the containing cyst, it is not possible to say with certainty. In the case described here, however, if the collapse is attributed to *Armullifer* infestation, then the reaction must be due to the escape of cyst fluid for the parasite must have been dead for some considerable time in order to calcify.



## CASE REPORT

A male Ibo native, E. N., aged 40 years, complained of the sudden onset of acute pain in the right side of the chest in the mid-axillary line. For 2 or 3 days previously he had complained of some fever but no other symptoms and no malaria parasites had been demonstrated in the blood. On examination, his temperature was found to be 103° F and respirations 36 per minute. His pulse rate was 100. There was impaired resonance in the mid-axillary line on the right side, while on the front of the chest below the clavicle was an area of hyper resonance. Breath sounds were absent in the mid-axillary line but on the front of the chest were tubular in character. Moist sounds were present below the clavicle. There was no finger clubbing. X ray examination of the chest (see X ray picture) showed complete collapse of the middle lobe of the right lung but no other abnormality in either lung. At the hilum of the right lung, however, was a small semicircular shadow with a definite corkscrew appearance. This was definitely a calcified nymph of *Armillifer*.

The patient was treated with sulphapyridine and made to perform breathing exercises. He made a satisfactory recovery and X ray examination 3 weeks later showed complete re-expansion of the lobe. The shadow in the region of the hilum remained unchanged.

## SUMMARY

A brief reference is made to recorded cases of infestation with *Armillifer armillatus* in which the parasite has been pathogenic. All these cases had been diagnosed at autopsy.

A case of pathogenic infestation with this parasite is recorded in which the patient made a satisfactory recovery.

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Collapse of right middle lobe with calcified nymph of *Armillifer armillatus*



TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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VOL 40 No 2 OCTOBER, 1946

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THE THIRTY-NINTH ANNUAL GENERAL MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1,

on

Thursday, 20th June, 1946

THE PRESIDENT

DR C M WENYON, C M G , C B E , F R S ,  
in the Chair

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BUSINESS.

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REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1946

The PRESIDENT, in presenting the Report (which had been circulated to those present), said the general conclusion was that we had had a very successful year and that the future offered an encouraging prospect for the Society

Dr NORMAN WHITE proposed the adoption of the Report This was seconded by Brigadier J A SINTON and carried unanimously

REPORT OF THE HON TREASURER FOR THE YEAR ENDED 31ST MARCH, 1946

The Hon Treasurer (Dr MARRIOTT) presented his Report, together with the Accounts and Balance Sheet prepared by the Auditors, Messrs W B KEEN & Co , and approved by the Audit Committee

He was very glad to report that Manson House had been entirely cleared of debt during the year. The Manson House Fund was, however, being kept open and any sums received would be devoted to expenditure on Manson House.

Dr MARRIOTT also referred to the increase in Fellows' subscriptions, to the record number of composition fees paid, and to the substantial sum of £409 received on letting the Lecture Hall. The Society's income was £5,366 an increase of £1,020 over the previous year and the excess of income over expenditure £819.

Dr C. C. CHESTERMAN proposed the adoption of the Treasurer's Report and, on being seconded by Brigadier BOYD, the motion was carried unanimously.

#### ELECTION OF THE AUDIT COMMITTEE

The PRESIDENT said that two members of the Audit Committee, Dr W. E. COOKE and Dr J. C. BROOM, were eligible for re-election but Dr C. R. AMES had gone to Egypt and it would be necessary to elect another Fellow other than a member of Council, to fill his place.

Sir PHILIP MANSON BAHR proposed the election of Dr C. A. HOARE as a member of the Audit Committee in place of Dr AMES and the re-election of Dr W. E. COOKE and Dr J. C. BROOM.

This was seconded by Dr GEORGE MACDONALD, and carried unanimously.

This concluded the business of the Annual General Meeting.

## ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1,

on

Thursday, 20th June, 1946, at 8 p m

THE PRESIDENT

DR C M WENYON, C M G, C B E, F R S,  
in the Chair

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## PAPER

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### RESEARCHES ON PALUDRINE (M 4888) IN MALARIA AN EXPERIMENTAL INVESTIGATION UNDERTAKEN BY THE L H Q MEDICAL RESEARCH UNIT (A I F)\*, CAIRNS, AUSTRALIA

BY

Brigadier N HAMILTON FAIRLEY, C B E, F R S, *et al*,

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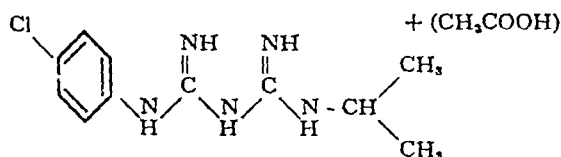
\* Director Brigadier N HAMILTON FAIRLEY C O and Senior Physician Lieut - Colonel C R BICKERTON BLACKBURN Entomologist Major M J MACKERRAS Pathologists Major T S GREGORY and Major J I TONGE Physician Captain R H BLACK Assistant Entomologists Lieut T H LEMERLE and Lieut Q N ERCOLE Assistant Pathologists Lieut K G POPE, Lieut S R DUNN, Lieut M S A SWAN and Lieut T A F AKHURST

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### INTRODUCTION

The brilliant researches of CURD, DAVEY and ROSE (1945) culminated in the synthesis of the biguanides M 4330 and M 4888 (paludrine) and the discovery of their causal prophylactic and therapeutic action in bird malaria. Both M 4430 and paludrine (M 4888) were found to exert a causal prophylactic and therapeutic action against different bird infections, but whereas M 4430 was found to be a causal prophylactic only against *Plasmodium gallinaceum* and to be without action on the blood forms of *P. cathemerium* (*P. relictum* not tested), no definite failure either in causal prophylactic or in therapeutic action was encountered with paludrine (M 4888) in birds infected with *P. cathemerium*, *P. gallinaceum*, *P. lophurae* and *P. relictum*. They concluded that these results justified the drugs being tried in causal prophylactic experiments and therapeutically with all types of human malaria. The chemical constitution of paludrine is given below



PALUDRINE

(Synonym, M 4888)

$\alpha$ -p-chlorophenyl- $\omega$ -isopropylbiguanide acetate

or

$\text{N}_1$ -p-chlorophenyl- $\text{N}_5$ -isopropylbiguanide acetate

In February, 1945, while in England on a military mission, one of us (N H F), had an opportunity of seeing something of the work being undertaken by CURD, DAVEY and ROSE at the Pharmaceutical Laboratories, I C I, Manchester. A month earlier therapeutic trials with paludrine in malaria-infected patients had begun at the Liverpool School of Tropical Medicine by ADAMS and his colleagues, and it was arranged through Dr C M SCOTT and the Medical Research Council that (1) researches on the suppressive and possible causal prophylactic action of paludrine should be undertaken on experimentally infected volunteers at the L H Q Medical Research Unit, Cairns, and (2) therapeutic trials be made on Australian troops infected with New Guinea strains of *P. falciparum* and *P. vivax*. Since then, supplies of paludrine adequate for these purposes have reached Australia regularly by air.



Over 200 volunteers have been used in experiments at L.H.Q. Medical Research Unit, Cairns, to determine the chemotherapeutic activity of paludrine on various stages in the life cycle of the malaria parasite. That New Guinea strains of *P. vivax*, *P. falciparum* and *P. malariae* were used in these experiments should be remembered when comparing therapeutic results obtained with strains from different countries.

The volunteers were all army personnel who were fit at the beginning of the experiments. None had served in malarial areas, in the South-West Pacific, or had previously suffered from malaria, jaundice, syphilis or asthma.

Five main investigations have been carried out by the L.H.Q. Medical Research Unit at Cairns to determine —

- (a) The value of paludrine as a suppressive agent and causal prophylactic.
- (b) The value of paludrine as a therapeutic agent (schizonticidal action).
- (c) The value of paludrine as a gametocide.
- (d) The mode of action of paludrine, with particular reference to possible effects on the pre-erythrocytic or hypothetical early exoerythrocytic (i.e.) forms.

- (e) Toxic effects arising from the administration of paludrine.

Extensive therapeutic trials in relapsing vivax malaria are also in progress at specially selected military hospitals on the mainland of Australia (vide Appendix, page 152).

## A. SUPPRESSION AND CAUSAL PROPHYLAXIS IN INITIAL EXPERIMENTS.

### 1. FALCIPARUM MALARIA.

Seventeen volunteers taking paludrine in various daily dosages were bitten by a variable number of anopheline mosquitoes (*A. punctulatus punctulatus*) containing viable sporozoites of New Guinea strains of *P. falciparum* in their salivary glands. The various batches used were from 60 to 100 per cent. infected, the infections in the salivary glands varied from light to heavy and the sporozoite age was from 3 to 12 days. Table I below sets out the detail of these groups with particular references to the degree of infection and the dosage of paludrine given for suppression.

It should be noted that in Group CIII biting only occurred on zero day and that paludrine was administered subsequently for 23 days (Chart 1). In the other groups the period of biting varied from 15 to 28 days and paludrine was administered daily throughout the period of exposure and for 23 days after the last infective bites.

Subinoculations of 200 c.c. whole blood into non immune recipients were performed on the 7th day after exposure from each of the six volunteers having 100 mg daily in Group CIII and from one having 25 mg daily. This was done to determine if erythrocytic parasites were appearing in the blood at the usual time.

Included in these groups were volunteers (1) taking atebirin (0.1 gramme daily), and (2) having no drug therapy. They were used as controls for the infectivity of the various batches of mosquitoes and for the subinoculations.

TABLE I

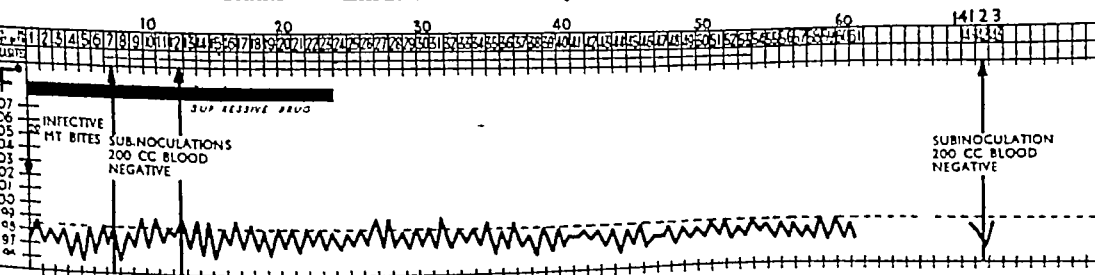
VOLUNTEERS USED TO TEST THE SUPPRESSIVE ACTION OF PALUDRINE AGAINST EXPERIMENTAL MOSQUITO-TRANSMITTED FALCIPARUM MALARIA  
(First Exposure on Day 0)

Group numbers	Number of volunteers	Paludrine			Total number of infective bites	Period of biting in days
		Dose in mg per day	Days administered	Total days of administration		
CIII	6*	100	-1 to +23	25	20	1
AWII	5	100	-1 to +42	44	41	15
AWIII	1	100	-1 to +42	44	42	15
AWIV	1	100	-1 to +53	55	6	26
AWIII	1	50	-1 to +42	44	42	15
AWIV	1	50	-1 to +53	55	6	26
AWIII	1	25	-1 to +42	44	42	15
AWIV	1	25	-1 to +53	55	6	26

Day 0 = day of exposure to infection Day -1 = day before exposure to infection  
Day +1, etc. = 1, etc., day after first exposure to infection

\* Subinoculations on the 7th day were negative in all six cases tested in Group CIII. Four control volunteers having atebirin (0.1 gramme daily) yielded positive subinoculations on the 7th day following last exposure to infection.

CHART 1—EXPERIMENTAL MOSQUITO-TRANSMITTED MALARIA



PATIENT P P L P *falciparum* (twenty infective bites on day 0)  
Paludrine (0.1 gramme daily), commencing 1 day before the infective bites and ceasing 23 days after the infective bites. Subinoculations were negative on the days 7, 12 and 142 following exposure to infection.

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## A. SUPPRESSION AND CAUSAL PROPHYLAXIS IN INITIAL EXPERIMENTS.

### 1. FALCIPARUM MALARIA.

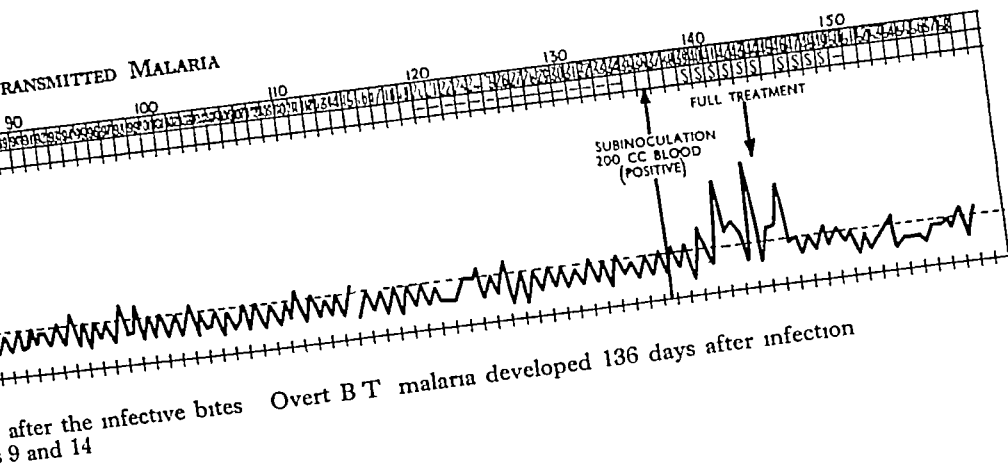
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HAMILTON FAIRLIE

TRANSMITTED MALARIA



3 days after the infective bites Possible radical cure

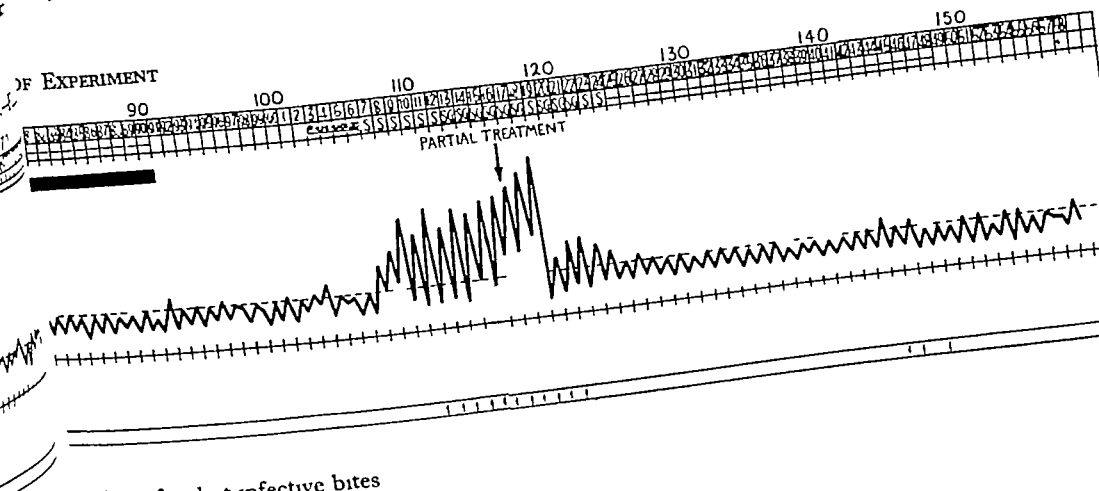
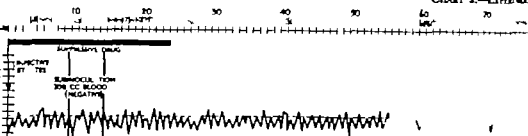
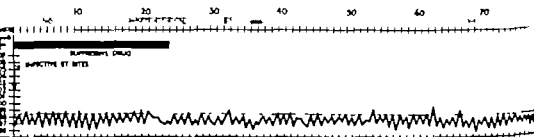


CHART 2.—EXPERIMENT

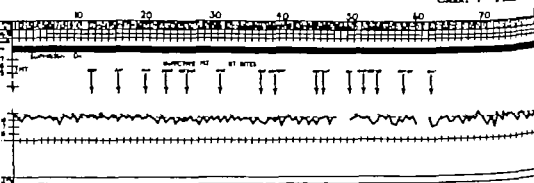


PATIENT L. P. T. *P. vivax* (twenty-one infective bites on day 0).  
 Paludrine (0.1 gramme daily) commencing 1 day before the infective bites and  
 Subcutaneous



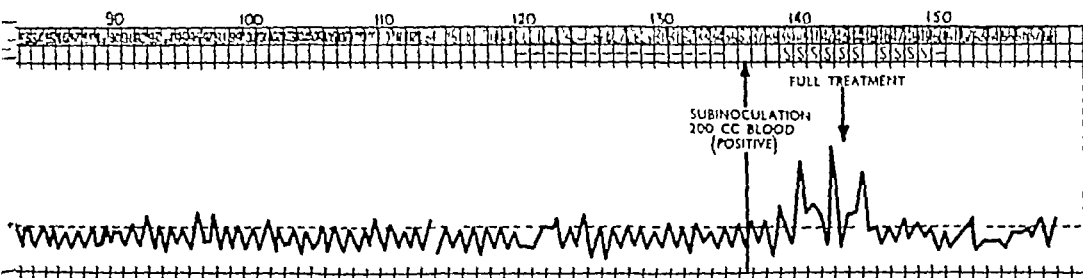
PATIENT N. W. S. *P. vivax* (twenty infective bites on day 0).  
 Paludrine (0.3 gramme daily), commencing 1 day before the infective bites and con

CHART 4.—FIELD TRI

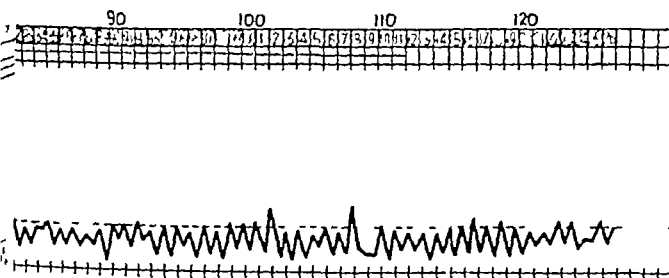


PATIENT E. J. C. 40 infective bites *P. falciparum* (123) and *P. vivax* (123).  
 Paludrine (0.1 gramme daily), commencing 1 day before first infective bites and con

## MOSQUITO-TRANSMITTED MALARIA

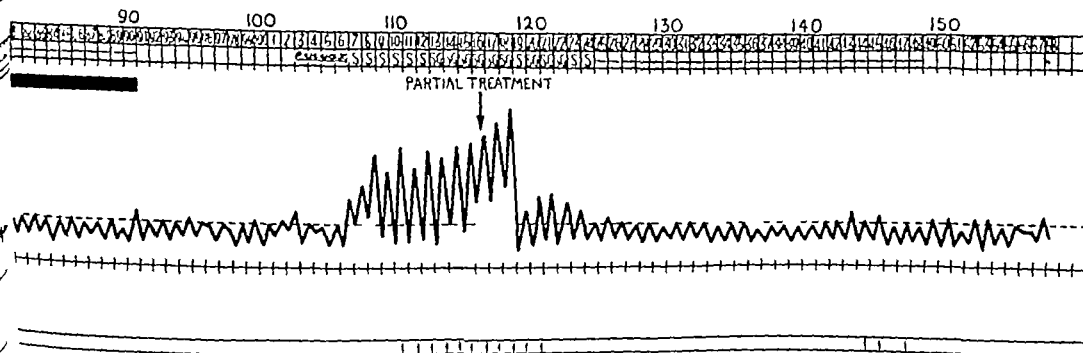


days after the infective bites    Overt BT malaria developed 136 days after infection  
 days 9 and 14



3 days after the infective bites    Possible radical cure

## OF EXPERIMENT



28 days after last infective bites

*Analysis of Results.**(a) During paludrine administration.*

(i) No volunteers having paludrine showed either demonstrable parasites in thick blood films (which were examined daily) or other evidence of overt malaria.

(ii) Seven out of the seven subinoculations performed on the 7th day after exposure to infection were negative, all seven recipients failing to develop malaria. On the other hand, the four volunteers having atebnin (grammes 0.1 daily), who were subinoculated on the 7th day after exposure, yielded positive results, all four recipients of their blood (200 c.c.) developing overt malignant tertian malaria.

(iii) Clinical features of suppressed malaria were invariably absent, and leucopenia and left shift of the polymorphonuclear leucocytes were not observed in the seventeen volunteers taking paludrine. On the other hand, minor clinical symptoms and left polymorphonuclear shift were present in the volunteers having atebnin.

(iv) The control volunteers having no suppressive drug therapy all developed demonstrable parasites and overt falciparum malaria within the normal incubation period, showing that the various batches of mosquitoes used were infective.

*(b) After ceasing paludrine administration.*

(i) Irrespective of whether they had received a daily dose of 25.50 or 100 mg. not one of the seventeen volunteers developed overt malaria or demonstrable parasites in thick blood films after ceasing paludrine.

(ii) The period of observation after ceasing paludrine administration varied from 40 to 119 days. In four instances subinoculations were performed on the 118th day after the last dose of paludrine—all of these were negative, the recipients failing to get malaria.

*Comments*

Exoerythrocytic or e.c. forms were first described by JAMES and TATE (1937) in reticulo-endothelial and endothelial cells in infected chicks (*P. gal. inaceum*) at a time when asexual parasites were present in the red blood corpuscles. It was in the same species of bird malaria (*P. gallinaceum*) some 7 years later that HOFF and COULSTON (1944) traced the development from the time the sporozoite entered the reticulo-endothelial system through the first generation of cryptozoites and the second generation of metacryptozoites to the production of asexual erythrocytic parasites. Though the pre-erythrocytic forms are exoerythrocytic and are probably identical with the e.c. forms

Throughout this paper unless otherwise stated, negative blood film refers to the absence of demonstrable parasites when 1 c.mm. of blood is examined as a thick blood film.

described by JAMES and TATE, there are advantages in the present state of knowledge in differentiating between the "primary tissue forms" or primary exoerythrocytic forms and the "secondary tissue forms" or secondary exoerythrocytic forms as suggested by DAVEY (1944). Neither stage has yet been satisfactorily demonstrated microscopically in man, but indirect evidence is accumulating which makes it difficult to accept any view other than that the pre-erythrocytic stage is present in *P. falciparum* and both the pre-erythrocytic and late exoerythrocytic forms in *P. vivax*.

In a small series of volunteers 25 to 100 mg of paludrine daily was found to act as a complete causal prophylactic against experimental mosquito-transmitted *P. falciparum* malaria (New Guinea strains). Negative subinoculation results, on the 7th day after exposure to infection, indicated that the primary wave of erythrocytic parasites from the pre-erythrocytic or early e e forms was delayed, inhibited or destroyed. Subsequent failure to develop demonstrable parasites or overt malaria when drug administration ceased indicated that destruction of the early e e forms had resulted. In the type of *P. falciparum* infection induced at Cairns, experimental results indicate that a negative subinoculation and failure to develop malaria some 6 weeks after ceasing drug therapy indicated complete causal prophylaxis.

The action of paludrine is fundamentally different from the action of atabrin, sontochn (SN 6911), resochin (SN 7618)\*, quinine and sulphadiazine. These latter drugs do not affect the early e e forms since positive subinoculations are consistently obtained on the 7th day after exposure to infection. Ultimate radical cure of the infection, however, generally follows the continuation of suppressive therapy with these drugs through schizonticidal action. In experimental sporozoite-induced falciparum malaria, early positive subinoculation and subsequent failure to develop overt malaria after ceasing drug therapy indicate suppression and radical cure by schizonticidal action.

As will be described later, paludrine in a dosage of 100 mg daily may be adequate for the cure of falciparum malaria if administration is commenced when trophozoites are present in the peripheral circulation and is continued for 14 days or longer. Thus, if paludrine failed to act as a causal prophylactic, there would be every prospect that it would still cure the infection by schizonticidal action, as does atabrin, sontochn, sulphadiazine or quinine when administered daily in appropriate dosage.

Further information on the true causal prophylactic action of paludrine in falciparum malaria will be found in Table V and Fig 1 (pages 129 and 130, and Table VI and Fig 2 (pages 132 and 133).

## 2 VIVAX MALARIA

Ten volunteers having paludrine were exposed to the bites of twenty mosquitoes (*A. punctulatus punctulatus*) with viable sporozoites of *P. vivax*.

\* Sontochn and resochin (chloroquin) belong to the 4 amino-quinoline group of compounds. They were patented in Germany—see D.R.P. 683692, November 13th, 1939—but were not subjected to extensive biological testing or adequate clinical study.



in their salivary glands. The batches of mosquitoes were 84 to 100 per cent. infective, the gland infections medium to heavy and the sporozoite age 3 to 10 days.

The volunteers were divided into two groups (Table II). The first, Group CI A, consisted of four volunteers receiving 100 mg paludrine daily (Chart 2, page 110), and the second, Group CI B of six volunteers receiving 300 mg daily (Chart 3, page 110). In both groups paludrine administration commenced on the day prior to exposure to infection and was continued daily till the 23rd day after exposure when the last dose was given. Thus the first group had a total dose of 2.5 grammes and the second group 7.5 grammes over a period of 25 days.

Subinoculations of 200 c.c. of whole blood were made from the four volunteers in Group CI A (receiving 100 mg paludrine daily) on both the 9th and 14th days after exposure to infective mosquitoes, *i.e.* eight subinoculations in all.

Included in these groups were volunteers either having no drug therapy or having stebrin (0.1 gramme daily) in suppressive dosage—these volunteers acted as controls.

TABLE II.

OCCURRENCE OF OVERT MOSQUITO-TRANSMITTED VIVAX MALARIA AND DEMONSTRABLE PARASITES IN VOLUNTEERS AFTER CELESTO PALEDRIKE ADMINISTRATION.  
(Single Exposure on Day 0.)

Group number.	Paludrine.		Number of volunteers exposed to infection.	Demonstrable parasites and overt malaria.	
	Dose in mg. per day	Duration of administration (days).		Number of volunteers.	Days since last dose of Paludrine.
CI A	100	-1 to +23	4	4	83 (range 37-117)
CI B	300	-1 to +23	6	1	19

Day 0 = day of exposure to infection. Day -1 = day before exposure to infection.  $xy + 1$  etc. = 1 etc., day after exposure to infection.

Four subinoculations were performed on the 9th and four on the 14th day after exposure to infection—all proved negative.

### *Analysis of Results*

#### *(a) During paludrine administration.*

(i) No volunteer in either of the two groups developed overt malaria or demonstrable parasites whilst having drug suppression—1 c.mm. of blood in thick films was examined daily.

(ii) All eight subinoculations from four volunteers having paludrine performed on the 9th and 14th days after exposure were negative. Subinoculation performed from a control volunteer having stebrin in Group

CI A was positive on the 9th day after exposure, but negative on the 14th day, the latter result being attributable to the schizonticidal action of atebryn.

(iii) Clinical features suggestive of suppressed malaria were absent or so mild as to have no significance in the volunteers receiving paludrine, leucopenia and left shift of the polymorphonuclear leucocytes were also not observed, but they were present in the volunteer having atebryn.

(b) *After ceasing paludrine administration* (Table II)

(i) Group CI A (100 mg daily) All four volunteers developed demonstrable parasites and overt malaria between the 37th and 117th days after the last dose of paludrine (average 82 days)

(ii) Group CI B (300 mg daily) One of the six volunteers developed demonstrable parasites and overt malaria 19 days after his last dose of paludrine. No clinical or parasitological evidence of vivax malaria was observed in the remaining five volunteers during a period of 70 to 118 days after ceasing paludrine administration. Evidence of delayed secondary attacks is awaited.

### *Comment*

Paludrine in dosage of 100 mg daily was adequate for the partial causal prophylaxis of experimental sporozoite-induced vivax malaria. With a dosage of 300 mg daily on the day prior to exposure, on the day of exposure and for the subsequent 23 days five out of six volunteers exposed to the bites of infective mosquitoes failed to develop evidence of active malaria in a period of 70 to 118 days after ceasing drug administration, i.e., 93 to 141 days after exposure to infection. In these volunteers paludrine may ultimately prove to have acted as a complete causal prophylactic, but no final opinion can yet be given as the period of observation is insufficient.

Subinoculations performed on the 9th day after exposure failed to reveal evidence of infection which indicated that the primary wave of erythrocytic parasites, presumably derived from the pre-erythrocytic or early e.e. forms, had been delayed. As all subinoculations made from volunteers having a daily dose of 100 mg of paludrine were negative, and as all four of these donors subsequently developed delayed attacks of overt malaria, it is evident that paludrine in this dosage was acting only as a partial causal prophylactic in these vivax infections.

### 3 FIELD TYPE OF EXPERIMENT

#### *(P. falciparum and P. vivax)*

A group of ten volunteers was used, eight received paludrine (100 mg daily), while two, having atebryn dihydrochloride (100 mg daily), acted as controls. Paludrine was administered on the day prior to exposure, on the

day of first exposure and for the subsequent 90 days—that is over the period of biting and for 28 days after the last exposure to infection (Chart 4 page 110).

The period of exposure to infection lasted 62 days. During this time there were sixteen biting seasons in which a total of 130 falciparum and 120 vivax infective bites were given to each volunteer by infected *A. punctulatus*. The infections, therefore, were exceedingly heavy and more intense than would ever be encountered in nature.

The batches of mosquitoes were from 64 to 100 per cent. infective, the infections in the salivary glands were medium to heavy and the sporozoite age varied from 1 to 10 days.

The experiment was designed as a field test and the following stresses and strains were imposed on the volunteers at different times —

(a) Heavy exercise—long marches in hilly country in a tropical climate were made, viz. 30 miles in 1 day 89 miles in 3 days and 72 miles in 36 hours. The climb in the longest marches was from sea level to 2,500 feet.

(b) Chilling—volunteers were exposed to extreme cold, viz., 1 hour at  $-10^{\circ}\text{C.}$ , with minimal clothing and restricted movement in a refrigerator belonging to the local meat works.

(c) Adrenalin (1:1000 solution)—0.5 c.c. was injected subcutaneously each hour for six to eight doses with the object of precipitating an overt attack of malaria if latent infection was present.

### *Analysis of Results*

#### *(a) During paludrine administration.*

(i) No volunteer developed overt malaria or minor clinical features suggestive of malaria—a left polymorphonuclear shift was never observed.

(ii) Parasites were never demonstrable in thick blood films from any volunteer having paludrine either during the period of exposure or during the subsequent 28 days before paludrine administration ceased.

(iii) No reactions to the various stresses and strains were observed that differed from those of the normal soldier.

(iv) No significant degree of anaemia or loss of weight developed—the average haemoglobin value before exposure was 16.8 grammes per 100 c.c. and after the last exposure 15.1 grammes per 100 c.c.

(v) No volunteer spent any time in bed as a result of malaria infection during the period of paludrine administration and no toxic features attributable to the drug were observed.

#### *(b) After ceasing paludrine administration*

(i) All the eight volunteers who had paludrine (100 mg. daily) developed demonstrable parasites (*P. vivax*) and subsequently overt vivax malaria 24 to 33 days after the last dose of paludrine was given.

(ii) No volunteer showed any evidence of falciparum malaria after ceasing paludrine administration, and parasites (*P falciparum*) were never demonstrated in thick blood films collected from these volunteers

(c) *During and after atebirin administration*

Suppression in the two volunteers was adequate while taking atebirin (100 mg daily) and the falciparum infection was radically cured by schizonticidal action of the drug. After atebirin administration had ceased both volunteers developed overt vivax malaria.

*Comment*

Volunteers repeatedly exposed to heavy infection by batches of mosquitoes infected with New Guinea strains of *P vivax* or *P falciparum* were protected against malaria attacks by a daily dose of 100 mg paludrine. Exercise to the point of physical exhaustion under field conditions did not produce any adverse effect on the volunteers taking paludrine and they reacted in a manner indistinguishable from that of normal soldiers.

Late results after ceasing paludrine administration confirmed the observations made in the experiments with *P falciparum* or *P vivax* infections alone, namely, that this drug is a complete causal prophylactic against falciparum malaria, but only a partial causal prophylactic against vivax malaria in a dosage of 100 mg daily when administered for limited periods of time.

4 GENERAL CONCLUSIONS REGARDING SUPPRESSION AND CAUSAL PROPHYLAXIS

(i) Paludrine administered in dosage of 100 mg daily prevented malaria developing in volunteers exposed to mosquitoes infected with New Guinea strains of *P falciparum*. Actually a dosage of 100 mg daily was continued for 23 to 28 days after the last infective bites, so that if *ee* forms had survived and produced erythrocytic trophozoites, cure would probably have been achieved by the schizonticidal action of the drug. In four volunteers receiving 25 to 50 mg daily, similar results were recorded.

(ii) Paludrine in a dosage of 100 mg daily prevents volunteers exposed to experimental mosquito-transmitted New Guinea strains of *P vivax* developing any symptoms of malaria or malaria parasites in the blood while this regimen is continued. This dosage acts as a partial causal prophylactic, but even if continued for 28 days after the last exposure to infection radical cure is not effected. Suggestive evidence of complete causal prophylaxis, however, has been obtained by administering 300 mg daily for 23 days after exposure to infection at a single session. Evidence is later cited suggesting that the prolonged administration of small doses of paludrine over many months may result in radical cure of *P vivax* infections.

(iii) A daily dose of 100 mg of paludrine is adequate for the protection

of volunteers repeatedly exposed to mixed vivax and falciparum malaria transmitted experimentally by mosquitoes even though the volunteers may be given most strenuous exercise and exposed to intense physical stresses and strains. After ceasing administration of the drug overt malaria develops, but this is caused by *P. vivax* never by *P. falciparum*.

(iv) Subinoculation results obtained at L.H.Q. Medical Research Unit, Cairns indicate that there is a fundamental difference between the mode of action of paludrine and plasmoquine on the one hand, and atebnin, santochn, resochin, sulphadiazine and quinine on the other in the protection of volunteers against malaria. With paludrine and plasmoquine the initial wave of erythrocytic parasites, originating apparently from schizogony of the early e.c. forms, is entirely prevented (*P. falciparum*) or delayed (*P. vivax*) with the other group of drugs erythrocytic parasites appear at the normal times, but are destroyed by the schizonticidal action of the drugs concerned provided they are present in adequate concentrations in the blood. The action of atebnin in this regard was reported to this Society last year by FAIRLEY and his colleagues (1945). Paludrine has the additional advantage over plasmoquine in being itself an excellent schizonticide.

## B. THERAPY—SCHIZONTICIDAL ACTION

### I. FALCIPARUM MALARIA.

MACGRAITH and his colleagues (1945-1946) reported having treated twenty two cases of falciparum malaria with paludrine ranging from 50 to 600 mg. every 12 hours for 14 days. The clinical response was satisfactory in all instances and no serious side effects followed the use of the drug. The subsequent follow-up proved difficult and it was not possible to determine the radical cure rate.

As previously reported from the L.H.Q. Medical Research Unit (FAIRLEY *et al.*, 1946), while the smallness of the dose of paludrine necessary to control a clinical attack was remarkable, even more remarkable was the finding at Cairns that this result might be achieved by administration of a small single dose of the drug. Thus in several cases of overt falciparum malaria with high parasite densities it was found that a single dose of 100 mg. of paludrine resulted in clinical cure and temporary disappearance of parasites as gauged by the examination of 1 c.mm. of blood in thick films stained by Field's method. Parasites however shortly reappeared and clinical recrudescences occurred.

While on an atebnin regimen of 0.1 gramme daily a similar result was noted during investigations at Cairns in two individuals who had both been infected with a relatively atebnin-resistant strain of *P. falciparum* acquired at Wewak. The plasma atebnin concentrations were found to be satisfactory notwithstanding, parasites of *P. falciparum* persisted in the blood in demonstrable numbers. In each instance the administration of a single dose of 100 mg. of

paludrine led to the rapid disappearance of parasites, they reappeared later in microscopic densities despite the continued administration of 0.1 gramme of atebirin daily

Two patients with naturally acquired *P. falciparum* infection received 100 mg of paludrine daily for 7 days—one was radically cured and the other developed overt malaria after cessation of the drug

In three out of three volunteers, experimentally infected with sporozoite-induced *P. falciparum* malaria, who were receiving 100 mg of paludrine from the 7th to 20th day, radical cure resulted. Infection was proved to have occurred, for subinoculations undertaken on the 7th day were positive, the two recipients developing overt falciparum attacks

The standard course of paludrine finally adopted for the treatment of overt falciparum malaria consisted of 100 mg thrice daily for 10 days (total = 3.0 grammes). The results of therapy in 105 cases of sporozoite- or trophozoite-induced falciparum malaria are given in Table III

TABLE III

RESULTS OF STANDARD THERAPY\* WITH PALUDRINE IN 105 CASES OF SPOROZOITE OR TROPHOZOITE-INDUCED FALCIPARUM MALARIA

Type of infection		Number of courses given	Secondary attacks (number recorded)	Response after the first day of therapy			
Experimental or natural	Sporozoite or trophozoite induced			Last day of temperature	Last day of symptoms	Last day of trophozoites	Last day of gametocytes
Experimental	Sporozoite	47	1	3	7	1	5—23+
Natural	Sporozoite	41	0	2	7	1	1—42+
Experimental	Trophozoite	17	0	2	5	1	10—38

\* Standard course of therapy consisted of 0.1 gramme of paludrine thrice daily for 10 days (total = 3.0 grammes)

Following the institution of standard treatment, it will be noted that trophozoites disappeared rapidly in the three series (1 day), that the temperature took 2 to 3 days to return to normal and that minor clinical symptoms persisted longer, i.e., 5 to 7 days. Though the trophozoites rapidly disappeared from thick films the gametocytes persisted for long periods despite treatment—up to 42 days in one instance

Seventeen out of seventeen trophozoite-induced infections, forty-one out of forty-one natural sporozoite-induced infections and forty-six out of forty-seven experimentally induced sporozoite infections were radically cured by this standard course of treatment

*Comment*

The over-all radical cure rate exceeded 99.0 per cent, with the standard course of 100 mg of paludrine thrice daily for 10 days. Significant toxic symptoms attributable to the drug were not observed in a single instance. From the standpoint of radical cure these results could hardly be bettered, and in our experience are superior to those obtained with any other anti malarial drug used for a similar period.

Clinical cure was rapidly obtained even with very low dosage and there was little difference between the response to the various courses of treatment enumerated above. The clinical response was not rapid and symptoms persisted longer than demonstrable trophozoites in thick blood smears. Trophozoites were cleared from the peripheral circulation within 2 days in the great majority of instances. A single dose of 100 mg given to volunteers with overt malaria and numerous parasites, rapidly rendered blood films negative in 1 c.mm. and there was steady if somewhat slow improvement in the clinical condition until they felt well. However this condition was temporary for parasites generally reappeared later and overt malaria again developed.

## 2. VIVAX MALARIA.

Certain features have to be appreciated in assessing the therapeutic response to paludrine both in natural infections and in volunteers experimentally infected with New Guinea strains of *P. vivax*.

In the first instance, the New Guinea strains of *P. vivax* used in experiments at Cairns have been found to behave somewhat differently from many of the strains previously worked with experimentally in other parts of the world. Following the primary attack of moderate or severe sporozoite-induced vivax infection, it is the rule with New Guinea strains for the first relapse or secondary attack to supervene some 6 weeks after the infection, and for the next attack to occur some 12 to 14 weeks after infection. Subsequent attacks were more variable and there appeared to be longer intervals of freedom. The number of sporozoites inoculated may be one factor in determining the time incidence of the second attack in subinoculation experiments decrease in the number of sporozoites injected tends to prolong both the incubation period and the interval between primary and secondary attacks. Less information is available on secondary attacks following the primary attack of trophozoite induced vivax malaria here the longest period of freedom observed has been 6 weeks.

At the L.H.Q. Medical Research Unit, Cairns, volunteers were examined clinically before infection and every day thereafter. It follows that the clinical criteria and progress of the infection under such conditions can be more accurately determined in experimentally infected volunteers than in naturally infected patients in routine hospital practice.

Similarly the standards of parasitological investigation were much more exacting. Actually 1 c.mm. of blood (approximately 1 000 high power fields

of thick blood films) was examined microscopically in each case before a negative report was made, this represented a much more thorough search for parasites than could possibly be made in laboratories doing routine blood films for malaria parasites. At the Liverpool School of Tropical Medicine MAEGRAITH and his colleagues (1946), in their therapeutic studies on patients treated with paludrine, examined 50 to 100 high-power fields for parasites in thick films.

The following courses of paludrine have been used for the treatment of volunteers with overt vivax malaria —

Course I Paludrine 10 gramme daily for 14 days Total of 140 grammes (Course commenced with either 10 gramme daily, or, if there was vomiting, with 200, 300 or 500 mg daily for the first 3 days and then 10 gramme daily for the next 13 days)

Course II Paludrine 300 mg daily for 14 days Total of 42 grammes

Course III Paludrine 300 mg daily for 10 days Total of 30 grammes

Course IV Paludrine 100 mg for 1 day Total of 0.1 gramme

The various groups of men treated have included volunteers with experimental sporozoite- or trophozoite-induced malaria and soldiers evacuated from New Guinea with naturally acquired malaria. Table IV sets out relevant data concerning the results of therapy with paludrine.

TABLE IV

RESULTS OF THERAPY WITH PALUDRINE IN SPOROZOITE AND TROPHOZOITE-INDUCED VIVAX MALARIA.

Number of course	Total paludrine (grammes)	Type of infection		Number of courses given	Secondary attacks (number so far recorded)	Response after the first day of therapy		
		Experimental or natural	Sporozoite or trophozoite induced			Last day of temperature	Last day of symptoms	Last day of parasites
I	140	Experimental	Sporozoite	43	6	4	8	5
		Natural	"	17	—	2	5	4
II	42	Experimental	"	4	2	3	10	6
II	30	"	Trophozoite	4	0	2	5	5
IV	0.1	"	Sporozoite	8	—	5	8	7
		"	Trophozoite	2	1	3	6	8

\* Considerable difficulty was experienced in differentiating female gametocytes from degenerating trophozoites affected by paludrine. For this reason clearance of the peripheral blood refers to disappearance of metocytes as well as trophozoites in thick films.

### Comment

(1) *Clinical Response* The clinical response to therapy was not rapid, in that the temperature did not reach and remain normal (±e, below 99° F) for some 3 to 6 days in volunteers treated for experimental sporozoite-induced



malaria they were not feeling really fit for some 7 to 11 days after commencing therapy. Little significant difference was noted between the response to the different courses of therapy. Volunteers with experimental malaria, however, were allowed to have overt malaria with high temperatures for several days before therapy was commenced. Treatment was seldom initiated until they had been confined to bed for 2 to 4 days. On the other hand, ordinary soldiers admitted with natural infections were treated as soon as the diagnosis of malaria was established. They were not allowed to have prolonged fever before receiving therapy and their therapeutic response was more rapid.

(ii) *Parasite Clearance* Parasites were cleared from the peripheral circulation within 5 to 8 days of commencing therapy. After 24 to 48 hours the parasites were either degenerate trophozoites or gametocytes. As remarked previously difficulty was experienced, especially in thick films, in differentiating female gametocytes from degenerate trophozoites, so it was not possible to determine with certainty when asexual parasites finally disappeared. In Table IV the rate of clearance of parasites appears to be partly related to the initial dosage of paludrine as the slowest clearance was observed in those volunteers receiving a single dose of 100 mg. paludrine. However it has been generally observed that the greater the parasite density at commencement of therapy the more the time required before the peripheral blood is cleared.

The response to paludrine of vivax infections acquired in India, Burma and the Mediterranean area has been investigated by ADAMS and MARGRAITH (1945) and MARGRAITH and his colleagues (1945-1946). Clinical cure was obtained in 147 cases of acute vivax malaria treated with paludrine in doses ranging from 10 to 700 mg. given every 12 hours for 14 to 28 days. No serious side effects of the drug were observed. In most cases the asexual parasites are stated to have disappeared from the blood by the 4th day of treatment and the sexual parasites by the 5th day. There was rarely more than one rise of temperature above normal subsequent to the beginning of treatment. The clinical and parasitological response to paludrine observed by MARGRAITH and his colleagues (1945-1946) appears to have been somewhat more rapid than those at Cairns. This may be due to a number of factors such as difference in intensity of infection, variation in the strain of vivax parasite, or differences in the clinical and parasitological standards adopted.

(iii) *Radical Cure* As yet insufficient time has elapsed to assess the proportion of radical cures as determined by the occurrence or absence of secondary attacks. As is seen in Table IV six volunteers developed secondary attacks following therapy for sporozoite-induced vivax malaria even after 1.0 gramme of paludrine was given daily for 14 days. The period of freedom from malaria in these volunteers who developed secondary attacks after Course I varied from 29 to 86 days. Fever occurred between the 29th and 34th day. In seventeen naturally acquired vivax infections receiving Course I, no relapses have been revealed. Sufficient time, however, has not elapsed to assess the significance of these results.

Two out of four volunteers with sporozoite-induced vivax malaria, who received 300 mg of paludrine daily for 14 days (Course III), have developed secondary attacks

One volunteer, treated with a single dose of 100 mg paludrine (Course IV) for overt vivax malaria induced by the inoculation of trophozoites developed a subsequent attack, another volunteer so treated has not relapsed for 66 days and may possibly prove to have been cured of his infection. The number of volunteers who have been treated for overt trophozoite-induced vivax malaria is too small to warrant any detailed analysis of results. No secondary attacks, however, have been recorded following the administration of 300 mg of paludrine daily for 10 days (Course III) to four volunteers with overt trophozoite-induced vivax malaria.

### 3 MIXED INFECTIONS WITH VIVAX AND FALCIPARUM MALARIA

Various courses of therapy have been given for overt mixed malaria (i.e., due to both species of parasite (*P. falciparum* and *P. vivax*), but the total number of volunteers treated at Cairns is small. Five experimentally infected volunteers or naturally infected soldiers have been treated with Course III and two with Course I. The response differed in no way from that observed with either falciparum or vivax malaria alone—the type of response depended on whether vivax or falciparum parasites predominated.

Late results of therapy will probably correspond with the observations already recorded, namely, radical cure of falciparum malaria and clinical cure of vivax malaria.

Of forty-one soldiers from New Guinea who were treated for overt falciparum malaria with paludrine, 300 mg daily for 10 days, twelve developed overt vivax malaria within 19 to 33 days after receiving their last dose of paludrine. None, however, developed recrudescences of malignant tertian malaria.

### 4 QUARTAN MALARIA

Two volunteers with experimentally transmitted *P. malariae*—one with a sporozoite- and one with a trophozoite-induced attack—have been treated with paludrine. The volunteer with sporozoite-induced malaria received paludrine 10 grammes daily for 14 days, the one with trophozoite-induced malaria received paludrine 300 mg daily for 10 days.

The clearance of parasites from the peripheral blood was not rapid—5 days in the volunteer with sporozoite-induced malaria, and 10 days in the volunteer with trophozoite-induced quartan malaria. The clinical response was rapid, but neither volunteer was as ill as the average volunteer treated for vivax malaria, nor were parasite densities in the peripheral blood so great. Last temperature of 99° or over was recorded 3 to 6 days after commencing therapy.

Insufficient time has elapsed since the end of therapy to enable any opinion to be expressed on the possibility of radical cure.

## 5 GENERAL CONCLUSIONS REGARDING THERAPY AND SCHIZONTICIDAL ACTION.

(a) Paludrine is an efficient schizonticide for the clinical cure of experimentally infected volunteers or soldiers with overt B.T. or M.T. malaria due to infection with New Guinea strains of *P. vivax* or *P. falciparum*. Its outstanding merit as a schizonticide is its ability in non-immunes to resolve overt attacks of falciparum and vivax malaria when administered as one single dose of 0.1 gramme or more\*. This should enable overt malaria in native villages and epidemics of falciparum malaria occurring in native populations to be rapidly controlled by a single dosage regimen instituted at weekly intervals. Direct therapeutic effects would result from schizonticidal action, but in addition suppression and causal prophylaxis, related to the time of drug administration, would eliminate or modify a considerable proportion of fresh infections.

(b) The clinical response was not more rapid than the response to quinine, atabrin or ontochin and appeared to depend on the duration of overt malaria before therapy commenced and on the parasite densities in the peripheral blood at the time treatment started. From this viewpoint, volunteers, who were heavily infected and in whom treatment was often withheld for a variety of reasons, were more difficult subjects for therapeutic testing than were naturally infected troops from New Guinea.

(c) The clearance of trophozoites from the peripheral blood was rapid in falciparum malaria and less so in vivax malaria, where the exact time was not precisely determined owing to the difficulty of distinguishing degenerating pre-trophozoites and female gametocytes. The rapidity of clearance depended primarily on the density present at the commencement of therapy. In falciparum infections blood films were usually negative after 48 hours therapy—this was probably related to the normal behaviour of *P. falciparum* in man in that it disappears from the peripheral circulation to undergo schizogony and at this stage paludrine produces its effect.

(d) Radical cure of falciparum malaria was readily obtained by paludrine when given in a dosage of 300 mg. daily for 10 days—eighty-seven out of eighty-eight sporozoite induced infections and seventeen out of seventeen trophozoite induced infections being cured by this course of treatment.

(e) Radical cure of sporozoite-induced vivax malaria was not regularly obtained even when a course of paludrine 1.0 gramme daily for 14 days was given. At the present stage of the investigation some secondary attacks have been observed after each of the different courses used, but they are fewer than would have occurred had previous courses of therapy employing quinine, atabrin and plasmoquine been used. Radical cure of trophozoite induced vivax malaria was readily obtained by paludrine as with most other anti malaria drugs.

Though clinical cure may be obtained by as little as 0.1 gramme, larger dosage would naturally be indicated for treatment of an overt attack.

# C THE ACTION OF PALUDRINE ON GAMETOCYTES

The effects of paludrine on the gametocytes of *P. falciparum* and *P. vivax* have been investigated in considerable detail by Major M J MACKERRAS and Lieut Q N ERCOLE. Their results, which will be published in detail later, are summarized below

## 1 *P. falciparum*

(a) *Effect on gametocytes in the blood of the carrier*—When paludrine is given in a dosage of 100 to 300 mg daily to a gametocyte carrier no effect is observed on the number or morphology of the gametocytes in the blood

Gametocytes taken into the stomach of the mosquito in a blood feed derived from a carrier on the first day of paludrine therapy show normal exflagellation and fertilization. Travelling vermicles are also formed which are able to penetrate the gut wall and form oocysts. These oocysts, however, fail to grow normally, they gradually shrivel up and disappear or persist as small chitunized spots. Complete sterilization of the infection resulted in mosquitoes fed on a carrier 1 hour after the first dose of paludrine (0.1 gramme) had been administered.

If mosquitoes are fed on a carrier on the second day of paludrine administration the gametocytes fail to develop as far as the oocyst stage. This failure of development was not found to be irreversible, for after paludrine administration had ceased and the drug had been eliminated completely, the gametocytes, if present in sufficient numbers, again produced sporozoite-infection in the salivary glands of the mosquito.

The sequence of events appears as follows

No gut infection was recorded in mosquitoes fed on gametocyte carriers 2, 4, 5 and 6 days after ceasing therapy (300 mg daily for 10 days). Oocysts formed, but failed to grow in mosquitoes fed on the 7th, 8th and 10th day after ceasing therapy. From the 12th day onwards oocysts developed normally and sporozoites reached the salivary glands. Evidently traces of paludrine sufficient to kill the parasite were still present in the blood on the 10th day, but had disappeared by the 12th day after cessation of treatment.

Other observations showed that the rate of recovery of infectivity of the gametocytes varied with the amount of paludrine administered—the smaller the dosage the more rapid the return to normal infectivity. These observations are of considerable interest as they indicate that the biological test is more delicate than the chemical determination of the drug concentration in the plasma.

(b) *Effect of gametocyte production*—As with atabrin, experiments revealed that the duration and height of the gametocyte wave following paludrine administration could be associated with the duration and height of the preceding trophozoite wave. Only if given very early in the attack could gametocyte

production be affected by paludrine and then only because the primary trophozoite wave was checked before gametogony had begun.

(c) *Effect on the sexual cycle of paludrine ingested by infected mosquitoes*—Mosquitoes engorged partially on a normal person taking 100 mg. of paludrine daily and were then allowed to complete their feed on another patient with gametocytes in the blood. The control group fed only on the gametocyte carrier. In the controls there was a heavy gut and salivary gland infection, whereas in the mosquitoes, which had fed on blood containing paludrine oöcysts formed in the gut but died without growing and sporozoites completely failed to develop in the salivary gland.

In two experiments when the paludrine blood feeds were postponed until the oöcysts were 5 days old in the first experiment and nearly mature in the second, the subsequent sporozoite rate in the mosquito remained unaffected. As active nuclear division must have been occurring in the formation of sporozoites, it would appear that paludrine was either not penetrating the cyst, or if it did so failed to attain an adequate concentration.

### Conclusions

(1) In therapeutic dosage paludrine affects neither the morphology nor number of falciparum gametocytes already formed, nor directly inhibits their production. When paludrine was given sufficiently early in the attack to cut short the trophozoite wave, the gametocyte wave was naturally similarly reduced.

(2) Though ingested falciparum gametocytes may undergo exflagellation and fertilization and even reach the stage of encystment in the gut, development ceases at this point if paludrine be present in even a small quantity. Complete sterilization of the infection may result in mosquitoes receiving a blood feed as early as 1 hour after a dose of 100 mg. of paludrine has been administered.

(3) If falciparum gametocytes persist in the blood for a long enough period after paludrine administration ceases they may regain their infectivity once the drug has been completely eliminated. Full infectivity has been observed to be re-established by the 12th but not the 10th day after completion of a course of 300 mg. daily for 10 days.

### 2. *P. vivax*

(a) *Effect of paludrine on morphology and the number of gametocytes*—Paludrine produced no detailed effect on the morphology of vivax gametocytes detectable in stained films. In wet preparations prepared 5 days after therapy was instituted the gametocytes appeared in characteristically rapid motion. No evidence was observed of inhibited the entry of newly formed gametocytes or increase in the number of gametocytes on the 1st, 2nd, and 3rd day of treatment. To a lesser degree this phenomenon was found with quinine, atabrin and plasmoquine. Owing

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degenerating preschizonts to female gametocytes in thick blood films, counts of mature male gametocytes were found to be more reliable than total counts in studying carriers treated with paludrine.

(b) *Effect of paludrine on infectivity of gametocytes*—When mosquitoes were fed on vivax carriers receiving a single dose of 0.15 or 0.2 gramme of paludrine, exflagellation, fertilization and vermicle formation were observed to occur normally. The vermicules penetrated the gut wall and encysted, but further development was arrested at this point, all that ultimately remained were small, shrunken cysts containing a few clumps of pigment. One exception occurred in a batch of mosquitoes fed 4½ hours after the first dose of paludrine; here some growth was noted and two oöcysts attained half size. All oöcysts, however, became shrunken and some chitimized. Sporozoites failed to develop in the salivary glands.

In mosquitoes fed on the 2nd day of therapy vermicules formed, but in only one instance did oöcysts form, in those fed on the 3rd and 4th days vermicules were not detected and oöcysts failed to appear in the gut wall.

### Conclusions

- (1) Paludrine does not directly inhibit the formation of gametocytes or modify the morphological appearance of those already formed.
- (2) If mosquitoes are fed on carriers who have recently had paludrine, development may proceed up to the oöcyst stage, but owing to persistent effects of the drug all oöcysts eventually die. Complete sterilization of the gut infection in mosquitoes results where 150 mg of paludrine has been administered to a carrier.

## D. MODE OF ACTION OF PALUDRINE

### 1. SPOROZOITE-INDUCED FALCIPARUM MALARIA

#### (a) *Action on asexual parasites*

The results of therapy showed that the peripheral blood was usually cleared of parasites in 48 hours—ring forms were withdrawn to undergo schizogony in the normal fashion, but a fresh crop of ring parasites did not appear after 48 hours. This suggested an action on amoeboid parasites or on later stages in the schizogonous cycle.

#### (1) *Effects of paludrine on cultures of P. falciparum in vitro*

Capt R. H. BLACK\* made a special study of the action of anti-malaria drugs in glucose serum to which infected blood corpuscles (*P. falciparum*) were added. The drug was not added directly to the culture medium, but was administered in appropriate dosage to the individual from whom the serum

\* See this number of the TRANSACTIONS, page 163.

was subsequently derived. In this manner the *in vitro* conditions of the experiment more nearly approach those existing in the body.

In cultures containing paludrine the parasites developed as far as early schizonts. Progress was halted at the stage of dispersion of chromatin before any division had taken place. These forms then became vacuolated and swollen and subsequently degenerated. No rings of the second generation were seen. In control culture using serum derived from an individual who had taken no paludrine the normal schizogamous cycle was completed.

(2) *The effects of paludrine on asexual parasites of P. falciparum in vivo.*

Owing to the fact that in *P. falciparum* infection schizogony takes place in the internal organs and that usually only ring forms and gametocytes are found in the peripheral blood, there was little opportunity for studying the effects of paludrine on the early schizont stages in peripheral blood smears in man. However in one patient with a heavy *P. falciparum* infection treated with paludrine, small numbers of degenerate early schizonts were observed in peripheral blood smears. Similar degenerative changes in early schizonts were also observed in marrow smears from a patient with overt falciparum malaria obtained 52 hours after the commencement of paludrine treatment. It would appear therefore, that the action of the drug *in vivo* and *in vitro* was exerted on the early schizonts, nuclear division being interfered with and degenerative changes occurring in the cytoplasm of the parasites.

(b) *Action on pre-erythrocytic forms*

It will be remembered that in experiments described in Table I page 109 seventeen out of seventeen volunteers receiving 25 to 100 mg. of paludrine daily for a period of 23 to 53 days after exposure to sporozoite-induced falciparum malaria failed to develop overt malaria or parasites in the blood.

The present series of experiments were designed to confirm whether this result was attributable to causal prophylaxis, i.e. eradication of the infection in the pre-erythrocytic stage before parasites appeared in the blood or to later schizonticidal action with destruction of asexual parasites in the blood and invaded red blood corpuscles.

Results of subinoculation experiments at Cambray infected with *P. falciparum* have shown infective with great regularity 144 hours + after biting of paludrine administration was adjusted accordingly. Stages of the parasite were adopted at exact stages of the parasite erythrocytic forms.

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the infections in their salivary glands varied from medium-heavy to heavy, and the sporozoite age was from 2 to 13 days

Table V and Fig 1 set out the detail of results in eighteen of these volunteers where paludrine was administered in a dosage of 100 to 300 mg on more than 1 day. Some of these experiments duplicate those already described in Section A on suppression and causal prophylaxis

(1) *Analysis of Results in Volunteers who received  
Multiple Doses of Paludrine*

Essential data regarding these experiments are incorporated in Table V and Fig 1 (page 130)

TABLE V

THE ACTION OF PALUDRINE IN EXPERIMENTAL MOSQUITO-TRANSMITTED FALCIPARUM MALARIA  
EIGHTEEN VOLUNTEERS RECEIVING PALUDRINE ON MORE THAN ONE DAY  
(100-300 mg daily)

Group number	Number of infective bites on day "0"	Paludrine		Number of volunteers exposed	Subinoculation on 7th or 8th day (+ = positive) (O = negative)	Results
		Dose in mg per day	Duration of administration in days			
CXI A	20	100	-1 to + 6	3	O	Causal prophylaxis
CXI B	20	300	-1 to + 6	3	O	" "
CVII A	20	100	+7 to +20	3	+	Suppression & cure
CVII B	20	300	+7 to +20	3	+	" "
CLI	10	100	0 to +1	1		Overt malaria
CLI	10	100	+1 to +2	1		Causal prophylaxis
CLI	10	100	0 to +2	1	O	" "
CLI	9	100	0 to +3	1	O	" "
CLI	10	100	0 to +4	1	O	" "
CLI	9	100	0 to +5	1	O	" "

Day 0 = day of exposure to infection      Day -1 = day before exposure to infection  
Day +1, etc = 1, etc, day after exposure to infection

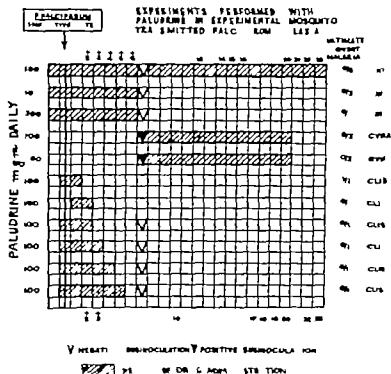
The results of these experiments are graphically depicted in Fig 1. In order to show how the dosage regimen contracted in successive experiments, the original experiment of 6 volunteers (Group C III) described in Section A on suppression and causal prophylaxis is included, but as it has been already considered in that section a further description of the results is unnecessary.

A perusal of Table V and Fig 1 shows the following results —

(1) Paludrine in a dosage of 300 mg daily administered from the day prior to exposure, on the day of exposure and for the subsequent 6 days, protected three out of three volunteers completely (CXI B). A similar group of three

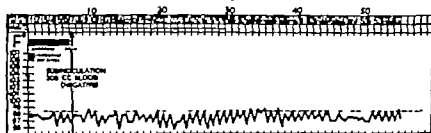


FIG. 1



volunteers (CXI A.) receiving 100 mg daily over the same time period, also showed complete protection for none ever developed parasites or an overt attack (Chart 5). Subinoculations made on the 7th or 8th day were also negative from volunteers in both groups, each of the six recipients of 200 c.c. of blood failing to develop malaria.

CHART 5—EXPERIMENTAL MOSQUITO-TRANSMITTED MALARIA.



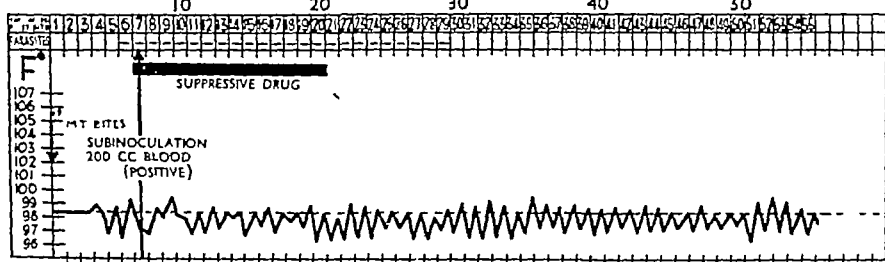
PATIENT F.B.S. *P. falciparum* (twenty infective bites on day 0).  
Paludrine (0.1 gramma daily) commencing 1 day before the infective bites and  
ceasing 6 days after the infective bites.

These results confirmed that paludrine in a dosage of 100 to 300 mg daily over this period was acting as a true causal prophylactic in falciparum infections. Paludrine in a dosage of 100 mg daily, given on the day of exposure and for the subsequent 2, 3, 4 or 5 days, fully protected four out of four volunteers (CLI Group). None of these volunteers subsequently developed malaria. The results indicated that paludrine was acting as a causal prophylactic in these dosages.

(2) One hundred mg of paludrine administered 3 hours before and 15 hours after exposure to infection (total of 200 mg) failed to protect the single volunteer exposed to infection, but 100 mg administered 15 hours and 39 hours after infection protected the single volunteer used on each occasion.

(3) One hundred mg of paludrine (Group CVII A) or 300 mg of paludrine (Group CVII B) administered daily from the 7th to 20th days after exposure inclusive, fully suppressed and ultimately cured six out of six volunteers—three on each regimen (Chart 6). Subinoculations made from each of these six volunteers

CHART 6—EXPERIMENTAL MOSQUITO-TRANSMITTED MALARIA



PATIENT W S S P *falciparum* (nineteen infective bites on day 0)  
Paludrine (0.1 gramme daily), commencing 7 days after the infective bites and  
ceasing 20 days after the infective bites

prior to commencing paludrine therapy were positive, and *P. falciparum* trophozoites were also demonstrated microscopically on one occasion to the order of 1 per c mm in two of these volunteers on the 8th day after exposure to infection. Cure in the six volunteers in these two groups was attributable to schizonticidal action of the drug.

## (2) Analysis of Results in Volunteers who received Single Doses of Paludrine

This experiment was undertaken at a late date after the war had ended. Volunteers were scarce and it was decided for the first time to permit officers and other members of the L H Q Medical Research Unit to volunteer for this purpose. Had this not been done this experiment could not have been completed. Table VI, Fig 2 (page 133), and Charts 7-12 (pp 135, 136) deal with the results obtained in fifty-four infected volunteers where paludrine

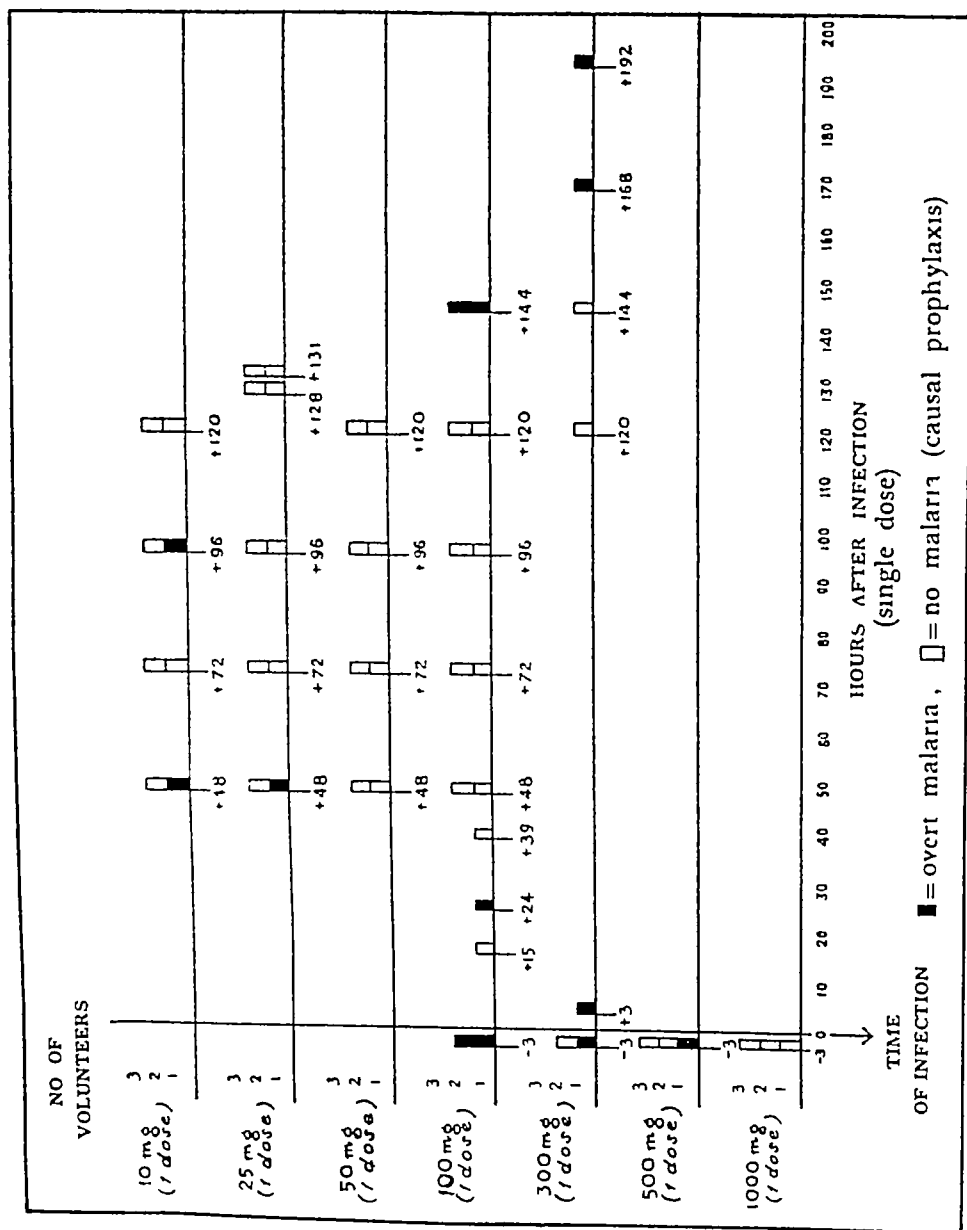
TABLE VI.

THE ACTION OF PALUDRINE IN EXPERIMENTAL MOSQUITO-TRANSMITTED FALCIPARUM MALARIA  
FIFTY-FOUR VOLUNTEERS RECEIVING SEVERAL DOSES OF PALUDRINE  
(all volunteers received 8-20 infective bites on day 0).

Time of single dose of paludrine. Before (—) or After (+) exposure to infection.		Dose of paludrine in mg.	Number of volunteers.	Results.	
Day	Hours.			Cerebral prophylaxis.	Overt malaria.
0	—3	1,000	3		
		800	3	3	0
		300	2	2	1
		100	1	1	1
	+3	300	1	0	2
				0	1
		100	1	1	0
		100	1	0	1
+1	+15 +24	100	1		
		100	1	1	0
				0	1
+2	+39 +48 +48	100	2	3	0
		80	2	2	0
		25	2	1	1
		10	2	1	1
+3	+72	100	2	2	0
		80	2	2	0
		25	2	2	0
		10	2	2	0
+4	+86	100	2	2	0
		80	2	2	0
		25	2	2	0
		10	2	1	1
+5	+120 +128 +131 +120	300	1	1	0
		100	2	2	0
		80	2	2	0
		25	4	4	0
+6	+144	10	2	2	0
		300	1	1	0
		100	2	0	2
+7	+168	300	1	0	1
+8	+192	300	1	0	1

Day 0 = day of exposure to infection. Day +1, etc. = 1st, etc., day after exposure to infection.

FIG 2



Causal prophylaxis in sporozoite-induced falciparum malaria following single doses of paludrine (10-1000 mg) administered at varying intervals from 3 hours before to 192 hours after exposure to infective bites (54 volunteers)

was given in a single dose of 10 to 1,000 mg. administered on one occasion only the time of administration of the drug varied from 3 hours before infection to 192 hours after exposure to infective bites (*P. falciparum*)

The results of this fundamentally important experiment are geographically depicted in Fig. 2.

(1) Paludrine, in a single dose of 1.0 gramme given on the day of exposure, 3 hours before biting, fully protected all three volunteers from falciparum malaria (Chart 7) 500 mg given at a similar time failed to protect one out of three volunteers (Chart 8) 300 mg given at the same time to two other volunteers failed to protect one of them (Chart 9), while 100 mg failed to protect either of the two volunteers who received their dose at the same time, i.e. 3 hours before biting (Chart 10)

(2) Paludrine, in a single dose of 300 mg on the day of exposure 3 hours after biting, failed to protect the single volunteer used.

(3) Paludrine, in a single dose of 100 mg given 15 hours after exposure, protected one out of one volunteer exposed, but administered 24 hours after exposure failed to protect the single volunteer who received this dose (Chart 11).

(4) Paludrine, in a single dose of 100 mg given 39 or 48 hours after exposure to infection, protected all three volunteers from falciparum malaria 50 mg administered similarly 48 hours after exposure protected two out of two volunteers doses of 25 mg and 10 mg., each administered to two volunteers 48 hours after exposure, protected one in each instance.

(5) 100 mg 50 mg., 25 mg and 10 mg of paludrine each administered as single doses to two volunteers 72 hours after exposure to infection fully protected all eight volunteers

(6) The experiment detailed in (5) was repeated, but the single doses were given 96 hours after exposure. Seven out of the eight volunteers were fully protected, one volunteer who received a single dose of 10 mg of paludrine developing overt malaria.

(7) An experiment as detailed in (5) was repeated, but the doses were given 120 (Chart 12) 128 or 131 hours after exposure one additional volunteer receiving a single dose of 300 mg of paludrine at 120 hours was included. All nine volunteers were fully protected, 10 mg apparently being as effective as 300 mg of paludrine.

(8) 300 mg of paludrine, 144 hours after exposure to infection, fully protected one volunteer but 100 mg failed to prevent overt malaria in two volunteers who received single doses.

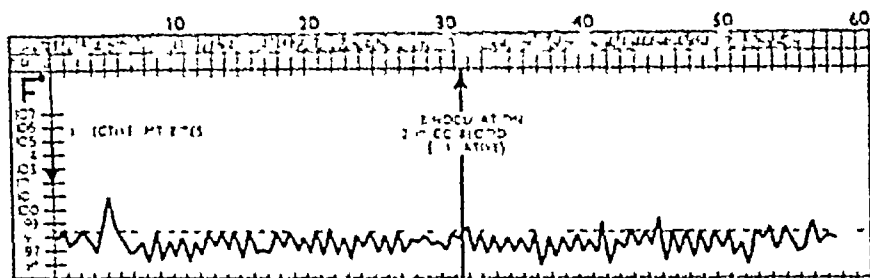
(9) 300 mg of paludrine failed to protect two volunteers who received their single dose 168 and 192 hours after infection

#### Comments

The results of these experiments confirm our previous finding that paludrine in appropriate dosage destroys the pre-erythrocytic forms of *P. falciparum* in

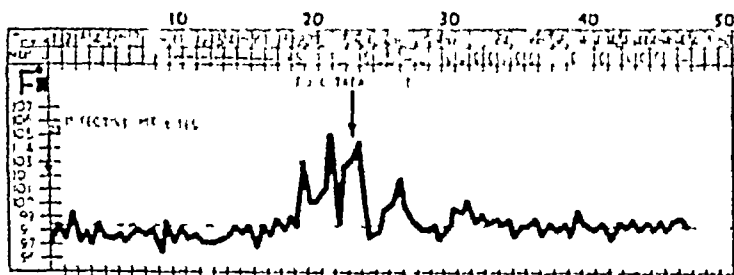
# EXPERIMENTAL MOSQUITO-TRANSMITTED MALARIA Simple dose relationship to causal prophylaxis

CHART 7



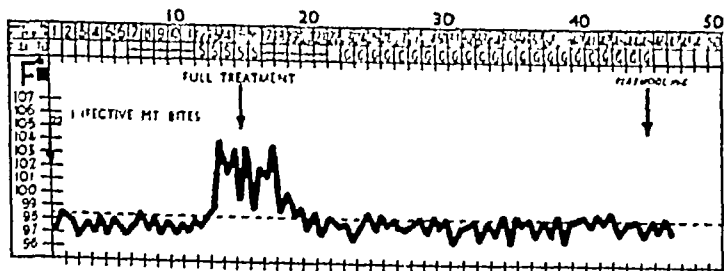
PATIENT F J J *P. falciparum* (ten infective bites on day 0)  
Paludrine (1.0 gramme), given 3 hours before exposure to the infective bite,  
acted as a true causal prophylactic, malaria failing to develop

CHART 8



PATIENT C K R A *P. falciparum* (twenty infective bites on day 0)  
Paludrine (0.5 gramme), given 3 hours before exposure to infective  
bites. Overt malaria developed 19 days after infection

CHART 9

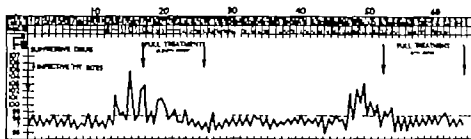


PATIENT J W S *P. falciparum* (twenty infective bites on day 0)  
Paludrine (0.3 gramme), given 3 hours before exposure to infective  
bites. Overt malaria developed 12 days after infection

# EXPERIMENTAL MOSQUITO TRANSMITTED MALARIA

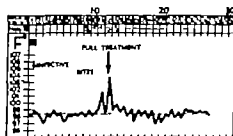
## Single dose relationship to causal prophylaxis.

CHART 10



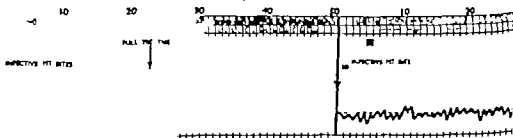
PATIENT: L. G. H. *P. falciparum* (ten infective bites on day 0).  
 Paludrine (0.1 gramme) given 3 hours before exposure to infective bites. Overt malaria developed 12 days after infection.

CHART 11



PATIENT: E. C. G. L. *P. falciparum* (ten infective bites on day 0).  
 Paludrine (0.1 gramme) given 24 hours after first exposure to infective bites. Overt malaria developed 11 days after infection.

CHART 12



PATIENT: C. H. R. A. First exposed to twenty infective bites on day 0 (*P. falciparum*). Paludrine (500 mg) was administered 3 hours before biting. Overt malaria developed on the 18th day. Radical cure followed malaria therapy. Subsequently the same patient was exposed to ten infective bites (*P. falciparum*) and 5 days later single dose of paludrine (100 mg) was administered. Overt malaria failed to develop.

True causal prophylaxis

volunteers exposed to experimental mosquito-transmitted falciparum malaria, with the result that asexual parasites fail to appear in the blood, *i.e.*, it is a true causal prophylactic.

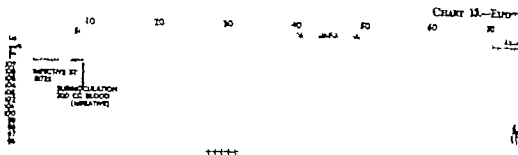
The failure of single doses of paludrine given 3 hours before exposure to afford protection in two out of two volunteers receiving 100 mg, in two out of three volunteers receiving 300 mg and in one out of three receiving 500 mg, is in marked contrast to the successful results obtained when as little as 10 to 100 mg were given as a single dose from 39 to 131 hours after infective biting. Such results indicate that sporozoites are far less susceptible to the action of paludrine than are the pre-erythrocytic forms of *P. falciparum*. Indeed, it appears extremely doubtful if the sporozoite is at all susceptible to paludrine, since the successful causal prophylaxis obtained in volunteers receiving larger doses (0.3 to 1.0 gramme) was probably due to its persistence in the circulating blood in concentrations sufficient to affect adversely the first generation of pre-erythrocytic forms (cryptozoites). It appears probable from the present researches that lethal effects by paludrine were produced by single doses of the drug as early as 36 to 48 hours after inoculation of sporozoites by mosquitoes, and that the first generation of pre-erythrocytes (cryptozoites) as well as the two subsequent generations (metacryptozoites) were highly susceptible to the action of this drug.

In the asexual erythrocytic cycle it will be remembered that paludrine has been found to interfere with the nuclear division at the preschizont or early schizont stage, these forms appear some 36 to 40 hours after invasion of the corpuscle by merozoites. If, as appears possible, there are three schizogonous cycles of 48 hours duration in the pre-erythrocytic stage of *P. falciparum* in man, paludrine may be acting on similar stages of the parasites in the pre-erythrocytic schizogonous cycle, *i.e.*, when nuclear division commences.

Since these pre-erythrocytic forms are so rapidly destroyed by a single dose of paludrine (10 to 100 mg) administered 2, 3, 4 or 5 days after exposure to infection, it follows that a tablet of 50 or 100 mg of this drug administered twice weekly, *i.e.*, on Wednesdays and Sundays, should be a complete causal prophylactic for *P. falciparum* infections.

The failure to cure all volunteers with sporozoite-induced overt falciparum malaria with courses of therapy consisting of paludrine 100 mg daily for 7 days, proves that the asexual erythrocytic forms were less susceptible than were the pre-erythrocytic forms of *P. falciparum* to the action of this drug. This was confirmed by the fact that a single dose of 10 to 100 mg of paludrine, given 5 days after exposure, protected volunteers, but when given in a dosage of 300 mg 7 or 8 days after exposure to sporozoite-induced falciparum infection, it failed to protect. In this later period, *i.e.*, 7 to 8 days after exposure, asexual erythrocytic parasites were demonstrated in the blood by subinoculation before paludrine administration had commenced.

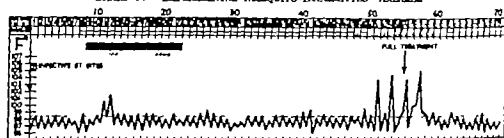




evidence of infection during a period of observation lasting 87 days (Group CIX A)

(4) 1 000 mg. paludrine administered for 14 days commencing 9 days after exposure, failed to produce radical cure in four out of six volunteers the other two volunteers showed no evidence of malaria infection during a period of 83 to 183 days observation (Group CIX B and Chart 14).

CHART 14 -- EXPERIMENTAL MOSQUITO-TRANSMITTED MALARIA



PATIENT C. D. C. P. (twenty-one infective bites on day 0).

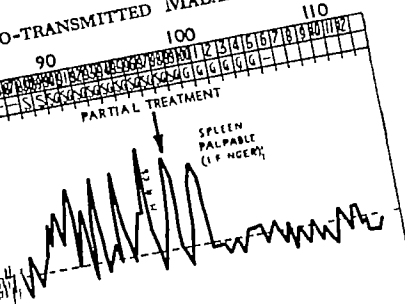
Paludrine (1.0 gramme daily) commencing 9 days after the infective bites and ceasing 22 days after the infective bites.

(5) Evidence of late attacks of malaria will be sought in all the volunteers mentioned above who may have been radically cured or in whom paludrine may have acted as a complete causal prophylactic. Subinoculations were performed from the two volunteers who did not develop evidence of malaria and who had received paludrine 1 000 mg daily from the day prior to exposure and for the subsequent 9 days. The recipients of 200 c.c. of whole blood collected on the 80th day after the donor had ceased paludrine administration did not develop either demonstrable parasites or evidence of overt malaria.

(6) Two volunteers who received either 1 000 mg on the day of exposure (Chart 15) or on the day of exposure and subsequent day (Chart 16), and two

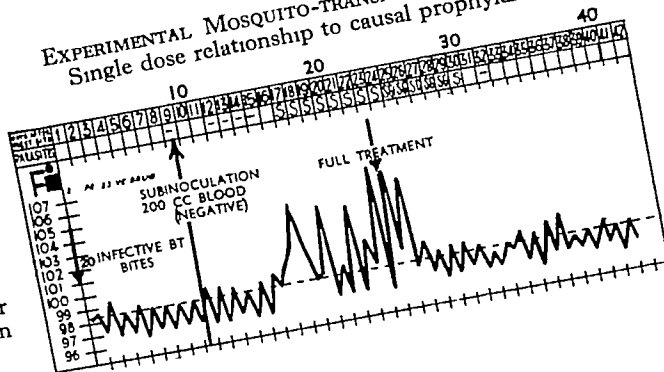
N HAMILTON FAIRLEY

## O-TRANSMITTED MALARIA



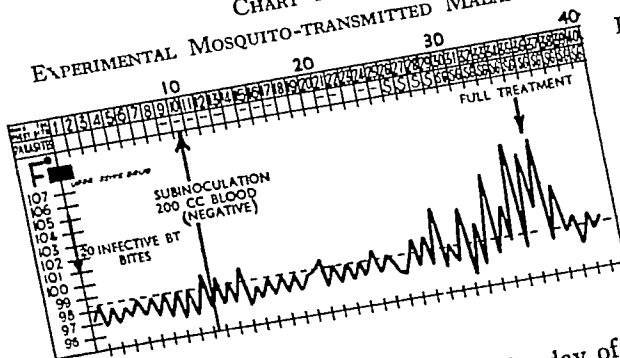
PATIENT A K A *P vivax* (twenty-two infective bites on day 0)  
 Paludrine (0.3 gramme daily), commencing 1 day before and ceasing 7 days after the infective bites  
 The onset of overt malaria was delayed to the 88th day

CHART 15  
 EXPERIMENTAL MOSQUITO-TRANSMITTED MALARIA  
 Single dose relationship to causal prophylaxis



PATIENT A A McP *P vivax* (twenty infective bites on day 0)  
 Paludrine (1.0 gramme), given 3 hours before exposure to infective bites  
 Overt malaria 16 days after infection, subinoculation negative on day 9

CHART 16  
 EXPERIMENTAL MOSQUITO-TRANSMITTED MALARIA



PATIENT T C *P vivax* (twenty infective bites on day 0)  
 Paludrine (1.0 gramme) for 2 days only, commencing on day of the infective bites, ceasing 1 day after the infective bites  
 Overt malaria 26 days after infection, subinoculation negative on day 10

volunteers, who received 100 mg on the day of exposure and subsequent 4 or 5 days, gave negative subinoculations on the 10th day after exposure. All four subsequently developed demonstrable parasites and overt malaria. In contrast to the findings in falciparum malaria, the conversion of a positive

into a negative subinoculation following treatment does not necessarily indicate cure in a vivax infection.

### *Comment.*

On the 9th or 10th day after exposure subinoculations were uniformly negative from volunteers who were either having paludrine 100 mg. daily or who had had 300 mg. daily from the day prior to exposure to the 7th day after exposure. This contrasted sharply with the uniformly positive findings in volunteers either having no drug therapy or who were fully suppressed with atebnin, santonchun resochin or quinine. Plasmoquine and M 4430\* were the only other drugs tested at Cairns that caused similar negative subinoculations. These subinoculation results indicated that the development of the primary wave of erythrocytic parasites had been inhibited—presumably by action either on sporozoites or pre-erythrocytic forms.

Negative subinoculations on the 10th day after exposure from volunteers who either had 1 000 mg. paludrine on the day of exposure or day of exposure and subsequent day and the similar findings from volunteers who had paludrine 100 mg. daily from the day of exposure to the 4th or 5th day afterwards, clearly indicate an action on pre-erythrocytic forms. The subsequent course of these volunteers and the other mentioned above has been the development of overt malaria. Thus paludrine acts as a partial causal prophylactic inhibiting but not completely destroying, the pre-erythrocytic forms.

### *3 Conclusions*

(a) In falciparum malaria paludrine is a true causal prophylactic as it has a definite lethal effect on the pre-erythrocytic forms, which are very susceptible a single dose of 50 to 100 mg. given 39 to 131 hours after exposure affords complete protection.

(b) In vivax malaria paludrine is a partial causal prophylactic in all instances and may in certain circumstances, be a complete causal prophylactic. It has an inhibitory effect on the pre-erythrocytic forms though this is less marked than in falciparum malaria, and complete eradication does not regularly occur.

(c) Paludrine is a powerful and true schizonticide which produces its effects on the early schizont and prevents chromatin division and formation of merozoites. This action is identical in vivax and falciparum malaria.

(d) Paludrine produces radical cure in falciparum infection with great regularity but the proportion of radical cures in vivax infection has not yet been established. There is evidence that better results may follow a smaller dosage over longer periods of time than a larger dosage over shorter periods. This is presumably due to more prolonged action on e. c. forms.

M 4430 in dosage of 200 mg. daily is partial causal prophylactic in vivax malaria.

## E TOXIC EFFECTS OF PALUDRINE

In the usual therapeutic dosage adopted, i.e., 300 mg for 10 to 21 days, significant toxic features were conspicuous by their absence. The toxic or possible toxic effects of paludrine that have been observed in experiments at Cairns with high dosage such as 10 grammes daily, may be divided into gastro-intestinal, urinary and haematological.

### (a) GASTRO-INTESTINAL EFFECTS

Vomiting commonly occurs in volunteers, or in patients with overt malaria, who are given courses of therapy beginning with 10 grammes daily. This vomiting appeared to be equally frequent, whether the 1st day's therapy was given as two doses of 500 mg or as five doses of 200 mg. However, if therapy commenced with doses of 100 mg thrice daily on the 1st day vomiting was more frequent than with other anti-malarial remedies, such as quinine or atabrin, administered to men sick with overt malaria. In one volunteer only was vomiting so severe that therapy had to be discontinued and fluids administered parenterally. Oesophagoscopy revealed some reddening and oedema of the lower end of the oesophagus which was probably related to regurgitation of acid from the stomach. In all other patients vomiting rapidly ceased when the daily 10 grammes dose was discontinued for 1 or 2 days. Two volunteers given 1,000 mg as a single dose (10 hours after the last meal) developed abdominal pain, vomiting and some diarrhoea for 12 hours.

### (b) URINARY CHANGES

These changes were associated with high dosage and were most marked in volunteers with overt malaria who were given 10 grammes daily for 14 days. The important findings were excessive numbers of red blood cells, sheets of epithelial cells probably arising in the lower renal tract, and a few hyaline or granular casts. At no time were changes observed suggesting that volunteers had nephritis rather than an irritative lesion of the renal pelvis with possibly some slight tubular damage.

One of the two volunteers given 1,000 mg as a single dose 10 hours after the last meal developed gross haematuria. The maximum amount (measured by haematocrit) of 12 per cent was reached 48 hours after his single dose of paludrine. Red cells to the order of fifteen to twenty high power fields were still present 18 days after having paludrine. No evidence of parenchymal damage to the kidney was observed. Subsequently he was given a course of paludrine 200 mg, 300 mg, 500 mg, and then 1,000 mg daily in divided doses for 13 days without incident.

Another volunteer developed haematuria, albuminuria, granular casts and some blood casts in his urine during a course of paludrine therapy consisting

of 1.0 gramme daily given in divided doses. At this time there was a raised blood urea concentration. This volunteer was considered to have had a pre-existing renal lesion not only because there had been transient albuminuria before his experimental malaria infection and the administration of paludrine, but also because a subsequent relapse of vivax malaria was associated with a similar aggravation of his renal syndrome though no paludrine had been administered.

Both the urinary and gastro-intestinal features were in general of minor significance—they occasioned no anxiety—were related to initial high doses of paludrine in volunteers sick with overt malaria, and were readily relieved by diminishing the dose of paludrine. In the majority of volunteers these phenomena disappeared within a few days even if the dosage was not reduced—disappearance of overt malaria appeared to play the major part in this.

#### (c) HAEMATOLOGICAL CHANGES.

Paludrine administered to normal volunteers in dosage of 1.0 gramme daily for 14 days produced a slight increase in the number of myelocytes—the average maximum was 1.15 per cent. of the total leucocytes. When 1.0 gramme daily was given to volunteers with overt malaria there was a transient but definite increase in total myelocytes with a maximum effect between the 7th and 10th days after commencing therapy—the maximum increase was to 10 per cent. of the total leucocytes. In infected volunteers treated with a single dose of 100 mg. of paludrine on 1 day only there was found to be a slight increase in myelocytes at the 7th to 10th day after commencing therapy. This observation was related to the subsidence of overt malaria rather than to paludrine administration as

- (i) No appreciable concentration of the drug would be present by the 7th day
- (ii) Previous observations had shown that this dose produced no increase in myelocytes in the normal volunteers and
- (iii) Similar changes had been demonstrated to follow therapy with atabrin and plasmoquine

It is considered that paludrine exerts a direct effect on the bone marrow which is more marked after an attack of overt malaria is treated. This effect is characterized by the transient appearance of myelocytes in the peripheral circulation. The degree of change is related to the dose of paludrine employed for therapy. In uninfected volunteers paludrine was not found to affect the total leucocyte count. Overt malaria itself tends to produce a relative leucopaenia, but no evidence of an additional decrease in the leucocytes or of increased tendency to agranulocytosis attributable to paludrine was ever noted.

#### F. GENERAL SUMMARY AND COMMENT

A series of experiments has been performed to determine the anti-malarial activity of paludrine in volunteers infected with New Guinea strains of *P. vivax*

and *P falciparum* Soldiers evacuated from New Guinea with overt vivax or falciparum malaria have also been used to determine its therapeutic efficacy

# 1 SUPPRESSION AND CAUSAL PROPHYLAXIS

Paludrine has proved superior to all known anti-malaria drugs as, in non-toxic dosage, it is a true causal prophylactic in falciparum sporozoite-transmitted malaria, and a partial causal prophylactic in vivax sporozoite-induced infections. The only other well-known drug which has a similar action is plasmoquine, but it has to be given in a dosage which is too dangerous for routine use for suppressive purposes.

In a dosage of 100 mg daily paludrine proved adequate for the full suppression of malaria in volunteers exposed to heavy infection by mosquitoes with viable sporozoites of *P vivax* or *P falciparum* in their salivary glands. Falciparum malaria was cured, but even when this dosage was continued for 23 to 28 days after the last exposure to heavy infection, vivax malaria later became overt. Subinoculation tests indicate that the primary wave of erythrocytic parasites arising from the pre-erythrocytic forms is inhibited in both vivax and falciparum infections, this being fundamentally different from the suppressive effects of atabrin, sontochin, resochin, quinine and sulphadiazine. With the latter drugs, suppression or cure is achieved by schizonticidal action, whereas with paludrine there is a direct effect on the pre-erythrocytic forms and asexual forms are destroyed and radical cure results (complete causal prophylaxis). In vivax malaria, with a regimen of 100 mg daily, the deleterious inhibitory effect is only temporary, evidently some of the pre-erythrocytic or early e e forms survive, and, after the administration of paludrine has ceased, commence schizogony with the eventual production of asexual erythrocytic parasites and overt malaria (partial causal prophylaxis).

In a field type of experiment, volunteers received a large number of infective bites (120 *P vivax* and 130 *P falciparum*) over a period of 62 days. During this time the men performed most strenuous route marches into the hills and while in the tropics were exposed to extremes of cold ( $-10^{\circ}\text{C}$ ). Injections of adrenalin and insulin were also given. While they were taking paludrine (100 mg daily) no parasites were ever demonstrated microscopically in the blood, and other schizonticidal drugs were absent and toxic drug effects were never observed. After ceasing to take the drug, overt vivax malaria developed in 24 to 33 days, but none ever manifested falciparum fever. Data collected during these and other investigations, summarized below, indicate that 50 mg of paludrine daily or 100 mg of paludrine twice a week, would constitute an effective causal prophylactic dosage regimen for non-immunes in hyperinfected areas of malaria in the tropics.

Experiments on seventy-two volunteers infected with sporozoite-transmitted falciparum malaria were designed to ascertain the effect of paludrine on different stages of the parasite, i.e., the sporozoites, the pre-erythrocytic forms and the asexual erythrocytic forms. In the first group of experiments paludrine, administered in a dosage of 100 to 300 mg daily from the day prior to exposure, on the day of exposure and for the subsequent 8 days afforded complete protection from nine to twenty infective bites (*P. falciparum*). Subinoculations on the 7th and 8th day were negative and overt malaria never developed. Here, paludrine was acting as a true causal prophylactic affecting either the sporozoite or the pre-erythrocytic forms.

In another group of volunteers paludrine in a dosage of 100 to 300 mg was given daily 7 to 20 days after exposure to infection (*P. falciparum*). Subinoculations on the 7th or 8th day were positive, but subsequently neither were parasites demonstrable nor did overt malaria develop. Here, cure was obtained by schizonticidal action after asexual parasites had gained access to the blood.

In a third group of volunteers (fifty four in number) exposed to infective bites (*P. falciparum*) paludrine was given in a single dose of from 10 to 1 000 mg. administered on one occasion only. The time of administration varied from 3 hours before exposure to 192 hours after exposure to infective bites. In general, it may be said that while a single dose of 100 mg. given 3 hours before exposure or more than 144 hours after exposure failed to protect, doses of 50 to 100 mg gave complete protection when administered 2, 3, 4 or 5 days after exposure. It follows that the pre-erythrocytic stage is more susceptible to paludrine than either the sporozoite or the asexual erythrocytic parasites, and that 50 to 100 mg administered twice weekly at 3 to 4 days interval, should afford complete causal prophylaxis against *P. falciparum* infections. Other experiments indicated that, while larger and repeated doses of paludrine completely destroyed asexual parasites this drug probably exerted no lethal action on the sporozoite, the protection afforded by larger doses (1 gramme) administered prior to exposure being due to its persistence in the blood in a concentration adequate to affect the first generation of pre-erythrocytic parasites, i.e., the cryptozoites.

## 2. THERAPEUTIC ACTION

Paludrine in non-toxic dosage controls malaria fever and terminates the clinical attack. It is a powerful schizonticide both in vivax and falciparum malaria. It acts on the early schizonts interfering with nuclear (chromatin) division. It produces radical cure in falciparum malaria, eighty-seven out of eighty-eight individuals with sporozoite-induced overt attacks and seventeen out of seventeen trophozoite induced overt attacks being completely cured by 300 mg daily for 10 days. Sporozoite induced vivax infections are not constantly cured even by 1.0 gramme daily for 14 days.

It is considered that a course of paludrine lasting 21 days, during which 300 mg would be given daily, would serve as an overall course for either vivax or falciparum malaria. This course would produce (1) radical cure of falciparum malaria, (2) clinical cure and a prolonged action on the hypothetical *ee* forms in vivax malaria, and (3) a period of at least 33 days, during which no infection of mosquitoes would occur. In all *P. vivax* infections and in the vast majority of *P. falciparum* infections, there would be too few falciparum gametocytes remaining in the blood to infect mosquitoes after 33 days had elapsed. Where individuals are not radically cured by such a course there are grounds for believing that a maintenance dose of 100 mg twice weekly for 6 months or longer would prevent relapses and eradicate the infection in a large proportion of individuals so treated.

### 3 DRUG CONTROL OF MALARIA IN INDIGENOUS NATIVE POPULATION

The outstanding merit of paludrine as a schizonticide is its ability to resolve overt attacks of falciparum or vivax malaria in non-immune volunteers when administered as a single dose of 100 mg or more. This should enable overt malaria in native villages as well as epidemics occurring in native populations, to be rapidly controlled by a single dosage regimen instituted at weekly intervals. Direct therapeutic effects would follow from schizonticidal action resulting in clinical cure and subsequent suppression of infection while causal prophylactic action would be exerted over a minimum period of 2 to 5 days after exposure to infection. These observations were made on non-immunes and it has yet to be ascertained in natives with premunity whether radical as well as clinical cure would result from such a regimen.

Acquired immunity in malaria appears to depend on the amount of antigen liberated and the duration of its action in stimulating hypertrophy of the reticulo-endothelium, specific opsonins or similar humoral antibodies and anti-toxic substances. The advisability of eliminating premunity by producing early radical cure of falciparum malaria in tolerant populations subject to repeated infections is open to serious doubt. Little or no immunity would result under such circumstances, residual immunity would soon disappear, the population would become highly susceptible and react severely when reinfected. This was the experience of KOMP and CLARK (1936, 1937), who treated all diagnosed infections in a highly endemic area in Panama with an intensive course of atabrin and plasmoquine, subsequently a severe outbreak of malaria supervened among the population so treated, while in adjacent villages, where therapeutic control of the attack and not radical cure had been attempted, no such epidemic occurred. SINTON (1939), who has made a special study of the effects of treatment upon the development and degree of immunity acquired in malaria infections, has clearly defined the general principles in relation to therapy as follows: (1) radical cure in populations of individuals



in whom the chances of reinfection are comparatively slight (2) rapid clinical cure of each attack, but not radical cure of the infection in populations liable to constant infection, reinfection and superinfection with multiple strains and species of parasite (3) clinical prophylaxis by means of an appropriate anti malaria drug when individuals are exposed only temporarily to chances of frequent infection and superinfection.

While these fundamentally important principles undoubtedly hold with anti-malarial drugs previously at the disposal of malarologists and clinicians, it is highly important that extensive investigations be undertaken in villages in malarious areas to ascertain (1) the effects of treating overt attacks of malaria in adults and children with single doses of paludrine (2) what single weekly dose regimen of paludrine will suffice to control clinical malaria without producing radical cure in infants, and in older native children and adults with premunity (3) what dosage regimen is necessary to produce radical cure in infants and native children and adults with premunity. Premunity in a native population is still bought at the exorbitant price of chronic anaemia, cachexia, splenomegaly with its attendant dangers and lowered resistance to intercurrent diseases, while the economic efficiency and productive capacity of such a community is greatly reduced. In view of the increased control of epidemics which paludrine and the use of new insecticides like DDT should afford, the pertinent question arises whether ultimately it would not be sounder policy in native villages where administrative facilities permit, to produce radical cure with paludrine and treat epidemics when they arise. As long as distribution and supplies of paludrine could be assured less damage to the community might result from loss of acquired tolerance following radical cure than the chronic ill health and the loss of energy and productive efficiency which characterizes communities afflicted with chronic malaria. This can only be finally determined by a long term policy of controlled field investigations extending over years. It is to be hoped that undue conservatism will not preclude wide scale trials of paludrine in native villages in hyper-endemic areas both with and without concomitant control measures directed to the eradication of the insect vector and its larvae.

Equally important are investigations of the effect of administering paludrine throughout the malaria season in countries like Macedonia where malaria transmission is strictly limited (May—October). Provided continuity of treatment could be assured each year malignant tertian malaria and black water fever might not only be controlled but eliminated by a regimen consisting of 100 mg. of paludrine twice weekly.

#### 4 THE ACTION OF PALUDRINE ON GAMETOCYTES.

When paludrine is given in a dosage of 100 to 300 mg. daily to falciparum or vivax gametocyte carriers, no effect is observed on the number or microscopic appearance of the gametocytes in the blood. Only if given very early in the

attack is gametocyte production affected by paludrine, and then only indirectly because the primary trophozoite wave is checked before gametogony had commenced

Though ingested falciparum or vivax gametocytes may undergo exflagellation and fertilization and even produce small oocysts, development ceases at this point and the oocysts die if paludrine be present even in small quantity in the blood feed. Complete sterilization of the gut infection in mosquitoes results some 1 to 2 hours after 150 mg of paludrine has been administered to a carrier. In mosquitoes fed on the 2nd day of paludrine therapy vermicules may form, but in one instance only did oocysts form (*P. vivax*), in those fed on the 3rd or 4th day of paludrine administration vermicules were not detected and oocysts failed to appear in the gut wall.

In carriers treated with paludrine, if falciparum gametocytes persist in the blood in sufficient numbers, they may regain their infectivity after the drug has been completely eliminated. Full infectivity has been observed by the 12th day after completion of a course of 300 mg daily for 10 days. Owing to the shorter duration of the vivax gametocyte wave, gametocytes are not found after a course of paludrine therapy in a vivax carrier.

## 5 TOXICITY OF PALUDRINE

The difference between the effective therapeutic dose and the toxic dose is very considerable and definitely exceeds that possessed by any other anti-malaria drug.

In the usual dosage regimen, such as 300 mg daily for 10 to 21 days, significant toxic symptoms were not observed.

Paludrine in a dosage of 1.0 gramme daily, when administered to volunteers with overt malaria, may produce toxic effects which are not serious and which may be relieved by diminishing the daily dose or by cessation of therapy for 1 to 2 days. The chief toxic effects observed have been vomiting and the presence of red blood cells, sheets of epithelial cells and occasional hyaline or granular casts in the urine. Gross haematuria developed in two instances with this dosage.

A transient increase in myelocytes in the peripheral circulation, which occurs on the 7th to 10th day after commencing therapy for overt malaria, has been frequently observed. Whether this is a direct toxic result of drug action or a specific stimulation by the drug of the myeloblastic tissue of the bone marrow recently released from the inhibitory effect of malaria infection, has not yet been determined.

When given as a suppressant drug, toxic symptoms have never been observed with a dosage of 100 mg for 3 months or 100 mg twice weekly for 6 months. A total of 21.0 grammes has been administered with impunity in 3 weeks by MÆGRAITH and his colleagues (1946). This amount equals the total dosage which would be given in 2 years on a regimen of 100 mg twice a week.

## G GENERAL CONCLUSIONS.

1. Paludrine is superior to all known anti malarial drugs as, in non-toxic dosage, it is a complete causal prophylactic in falciparum malaria.

2. It has a definite lethal action on the pre-erythrocytic forms of *P. falciparum* which are highly susceptible—thus, a single dose of 50 to 100 mg. given from 39 to 131 hours after exposure to infective bites, affords complete protection. Lethal effects are probably not exerted on the sporozoite which appears to be unresponsive to paludrine.

3. In vivax malaria paludrine acts as a partial causal prophylactic in all instances and asexual parasites fail to gain access to the blood while the drug is being taken. During this period subinoculation tests are negative. This suggests an inhibitory action on schizogony of the pre-erythrocytic or e.e. forms, but their complete eradication does not regularly occur and overt attacks are liable to follow cessation of the drug.

4. Paludrine is a powerful schizonticide which produces its effects on the early schizont and prevents nuclear (chromatin) division and the formation of merozoites. This action is identical in vivax and falciparum malaria.

5. Paludrine readily controls the clinical attack and produces radical cure in falciparum malaria with great regularity—eighty-seven out of eighty-eight sporozoite induced falciparum attacks and seventeen out of seventeen trophozoite-induced attacks of overt malaria were radically cured by a 10 days course of 300 mg. of paludrine daily.

6. In vivax infections clinical cure is also obtained, but neither the proportion of radical cures produced by paludrine therapy nor the best standard course of treatment has yet been determined. There are grounds for believing that better therapeutic results, i.e. a lower relapse rate, would follow a smaller dosage over longer periods of time than a larger dosage over a shorter period—a result presumably attributable to more prolonged action on e.e. forms.

7. Paludrine exerts no obvious primary effect on either the number or microscopical appearance of gametocytes in the blood of the carrier. The number of gametocytes will be affected only if the drug is given sufficiently early in the overt attack to terminate the primary trophozoite wave prematurely soon after the appearance of asexual parasites in the blood.

8. Though paludrine is not gametocidal in the carrier sterilization of the infection occurs in the gut of the mosquito vector fed on a falciparum or vivax gametocyte carrier as early as 1 to 2 hours after the first dose of the drug is taken. This sterilization effect persists while the drug is being administered and for a variable period thereafter depending on the dosage taken, i.e. until the drug has been eliminated from the body.

9. Paludrine is remarkably free from toxic complications and the difference between the effective therapeutic dose and the toxic dose is very considerable. Apart from individual idiosyncrasy to the drug of which no example so far appears to have been encountered, significant toxic symptoms are not likely

to follow its use in any dosage regimen adopted for purposes of therapy, suppression and causal prophylaxis

10 Its pre-eminent therapeutic achievement is that in non-immunes a single dose of 100 mg (1) may terminate a clinical attack of falciparum or vivax malaria, and (2) is a complete causal prophylactic if given from 2 to 5 days after exposure to infective bites (*P. falciparum*). As a complete causal prophylactic the dosage recommended is 100 mg every 3 or 4 days, *i.e.* twice weekly (Wednesdays and Sundays). The potentialities of paludrine for the chemotherapeutic control of malaria in the non-immune Europeans, as well as in indigenous native populations with premunity, are enormous, and call for controlled field investigations on a large scale

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## H. APPENDIX

INTERIM REPORT ON THERAPEUTIC TRIALS IN RELAPSING  
VIVAX MALARIA.(1) PALUDRINE TREATMENT FOLLOWED BY A MAINTENANCE DOSE  
(100 MG.) TWICE WEEKLY FOR 6 MONTHS.

As indicated elsewhere there are grounds for believing that radical cure in vivax infections is not merely dependent on schizonticidal action as in falciparum malaria but is related to lethal action of the drug in eradicating persisting e.e. forms which are responsible for maintaining the infection between relapses. If relapses are preceded by increased schizogony of e.e. forms, and if the e.e. forms are most susceptible when nuclear division is occurring, maximum therapeutic effects would be expected if the drug were present in the blood at such times. This might be better attained by prolonged administration with low dosage rather than by administration of a high dosage for a short period of time. Clinical trials were devised accordingly.

At 116th A.G.H. tuberculous patients and nursing sisters with relapsing vivax malaria have received an initial course of 300 mg. of paludrine daily for 3 weeks followed by a maintenance dose of 100 mg. of paludrine twice weekly which is being continued for 6 months. Toxic effects were never observed. The period of observation on these cases has been 5 months, and up to date Lt.-Col. H. W. WUNDERLY reports that no minor or major symptoms of malaria have developed and parasites have never been found in the blood smears. Before this, relapses at this hospital had been frequent both in the patients and the nursing staff. The results have been particularly satisfactory for previously many of the tuberculous patients had deteriorated after two bouts of fever while the relapses were so frequent amongst the sisters that it had been difficult to keep the staff up to strength. For reasons outlined above it is anticipated that the incidence of residual vivax infections, after this 6 months maintenance dosage has been completed will be small.

(2) A COMPARISON OF THE VALUE OF QUININE PLASMOQUINE AND PALUDRINE  
PLASMOQUINE TREATMENT IN THE RADICAL CURE OF RELAPSING VIVAX MALARIA.

As both paludrine and plasmoquine act as true causal prophylactics in falciparum malaria, and as subinoculation results indicate that both these drugs have a definite inhibitory action on the pre-erythrocytic parasites of *P. vivax* extensive clinical investigations using a combination of these drugs were instituted at the 112th, 113th and 115th Military Hospitals on the mainland of Australia. At these three centres a comparison in the clinical and radical cure of vivax malaria has been and is being made by the C.O.s of the Clinical Division of the efficacy of the British Army course of 10 days treatment with quinine (30 grams) and plasmoquine base (30 mg.) daily introduced by SINTOX, and a combined course of paludrine (0.3 gramme) and plasmoquine base

(30 mg) daily for 10 days. The total number already treated exceeds 1,400 cases (*P. vivax*). It is too early yet to assess the relative value of the two systems of therapy from the standpoint of radical cure, but over a variable period extending up to 6 months, both courses of treatment have proved superior to standard quinine, atebryn and plasmoquine treatment formerly adopted in the South-West Pacific. In 232 patients (Group A) receiving paludrine and plasmoquine and 223 patients (Group B) receiving quinine and plasmoquine under comparable conditions, the relapse rate to date has been 13.4 per cent and 13.5 per cent respectively. The mean number of previous courses of treatment was 1.77 and 1.82 in the two series. The average interval between treatment and relapse has been 66.5 days (extremes 25 to 130 days) with paludrine-plasmoquine treatment, and 31.4 days (extremes 11 to 88 days) with quinine-plasmoquine treatment. Differences in the rate of excretion or degradation of paludrine and quinine will not suffice to explain the disparity in time between cessation of treatment and reappearance of parasites in the blood smears in the two series. This appears to depend on the inhibiting action exerted by paludrine on the late c.c. forms in *P. vivax* infections.

Enhanced toxicity of plasmoquine has not been noted in the paludrine-plasmoquine series compared with the quinine-plasmoquine series when using 300 mg of paludrine and 30 mg of plasmoquine base daily. The troops generally prefer paludrine and plasmoquine to quinine and plasmoquine owing to the unpleasant taste of quinine and the nausea, vomiting, tinnitus or deafness sometimes arising from quinine administration. Cyanosis of varying degree due to methaemoglobinæmia was encountered in both series, but out of approximately 1,400 patients receiving plasmoquine (30 mg daily) combined with either quinine (30 grains daily) or paludrine (0.3 gramme daily) for 10 days, the full course of treatment has been completed in all but two instances. These were both Australian Army nursing sisters with *vivax* malaria who were receiving quinine-plasmoquine treatment, nausea and vomiting proved so severe that a change to atebryn therapy was made.

Lieut.-Colonel C. R. BICKERTON BLACKBURN, in collaboration with Major WITHERS, is following up the subsequent history of these three groups of patients in Australia, the final results should afford information of considerable value regarding the incidence of radical cure in *vivax* infections.

## DISCUSSION

The President, Dr C. M. Wenyon. We are very much indebted to Brigadier FAIRLEY for his most interesting account of the successful investigations carried out at Cairns under his directions. It seems to me that the information he has given us this evening supports very strongly the conclusions he previously arrived at, namely, that the difference between the malignant tertian parasite and the benign tertian parasite from the point of view of the

persistence of infections, can best be explained on the basis of differences in the endothelial development. It would seem that in the case of *P. falciparum* the hypothetical endothelial stage following inoculation of sporozoites passes through only two or three cycles which occupy 6 or 7 days, whereas in the case of *P. vivax* the endothelial stages persist for many months, or even 2 or 3 years, with an indefinite number of cycles. If this assumption is correct it may explain why it is so very difficult to treat benign tertian malaria and finally eradicate the infection. It seems evident from what we have been told that in paludrine we have a drug which will cure malignant tertian malaria. Atabrin is able to do this but not quite so easily. In the case of benign tertian malaria paludrine has proved more successful than any other drug, and brought us much nearer to its permanent suppression. Brigadier FAIRLEY has told us that attacks of benign tertian malaria can be successfully treated with paludrine and that if a maintenance dose of 100 or 200 mg. a week is continued relapses of benign tertian malaria do not occur.

It does seem probable that it is now possible not only to treat attacks of benign tertian malaria satisfactorily but also to do away with the annoyance of repeated relapses.

I will not take up any more time as I am sure there are many here who would like to comment on what Brigadier FAIRLEY has told us or ask him to explain more fully any doubtful points.

Brigadier J. A. Sinton. I consider the Fellows of our Society are very fortunate in having heard from Brigadier FAIRLEY twice during the last 18 months such important reports on the chemotherapy of malaria. We have reaped one of the few benefits of the war in that the Australian Forces have made possible and have carried out researches the results of which would have taken decades to obtain under peace. It gives me great pleasure to speak about the research work. I was fortunate enough to see the laboratory research and the dramatic results of its application in the field. Brigadier FAIRLEY has only considered a very small portion of the investigations carried out. I think the thanks of the tropical world are due to Brigadier HAMILTON FAIRLEY and his team for the magnificent work they have done.

This new work on paludrine is especially one of which I think the potentialities are enormous. We have also to thank the L.C.I. workers who have produced this drug for investigation. Scientifically paludrine appears to open up new fields, especially that of causal prophylaxis. Previously we had no drug except plasmoquine, which the late Colonel JAMES found had a true causal prophylaxis but unfortunately in doses too high for ordinary therapeutic use. We are not in a position to say whether or not paludrine will prove to be a panacea for the malaria of the world, but the prospects are very encouraging. It will be of great value among the non-immune populations, such as the armies and Europeans in the tropics and, apart from those the possibilities

among the indigenous semi-immune appear to be tremendous. The clinical manifestations in such populations, which form the bulk of the tropical peoples, are more easily controlled than are those among the non-immunes such as the Army sent into the tropics. It is probable that relatively small doses given at longer intervals may control clinical manifestations in the former type of population and it may be found practicable to go round villages once a week and give the majority of people a tablet to swallow. We are getting nearer what I would consider the ideal malarial drug for suppression, *i.e.*, one of which a single dose would protect for many months. With regard to giving this drug to such populations paludrine has again another great advantage, in that the relationship between the optimum therapeutic dose and the toxic dose is very wide. One can therefore give a dose of a relatively harmless nature to these people without, so far as we know, any risk of producing undesirable symptoms. That is very important, because, if unpleasant or alarming effects are produced among ignorant people, you will make your drug unpopular and an undisciplined population will not take it.

Some later speaker may be able to tell me whether there is any deterioration in the potency of the drug, or enhanced toxicity under varying conditions of storage, such as the great ranges of temperature and humidity met with in the tropics where sometimes drugs are stored for long periods to meet emergencies.

I would ask Brigadier FAIRLEY what he thinks about the dosage to be recommended for non-immune populations, such as the Army or European people in malarious areas, also what for the semi-immune ordinary indigenous population of the tropics? As Brigadier FAIRLEY pointed out, it is interesting that the drug has proved to be a true causal prophylactic in M T malaria and also acts against the *e e* forms of *P. gallinaceum* but *not* against the hypothetical ones of benign tertian. This again shows that, while experiments in birds may give some guide as to what a drug will do, they give no certain indication of how it will act against all the different types of human malaria. I was pleased to note that Brigadier FAIRLEY spoke of the primary tissue stages between sporozoites and the primary erythrocytic forms as pre-erythrocytic, in contradistinction to the secondary tissue forms commonly called *e e* forms. It is often assumed that those two forms are identical, but we do not know that they exist in mammalian malaria, still less that they are identical.

There are certain questions I would like to ask Brigadier HAMILTON FAIRLEY about atebryn. What are the advantages of paludrine over atebryn and all the other drugs, more especially as a suppressor? Does he consider we are now in a position to replace atebryn with paludrine if supplies are available? Also, whether he thinks better results are to be achieved by giving either atebryn or paludrine in very large doses over a short period rather than by smaller doses over a longer period? From my experience with other anti-malarial drugs, I consider that probably better results can be obtained, not by giving



very large doses for 3 or 4 days, but by smaller doses for 10 or 20 days but I would like his opinion on this point.

I understand that we must not go away with the idea that there were large numbers of these so-called "stebrin resistant" cases present at Wewak, but that the numbers were so few that they did not detract from the great value of stebrin in the field. They were confined to a very small proportion of those people who had been taking the drug in a very irregular manner.

Before I close I would again like to pay a tribute to the Australian Army. I saw their anti-malarial work in the Solomon Islands, New Guinea, and other places. It was astonishing in the Solomon Islands to go there and see, in places where 70, 80, 90 or 100 per cent. of the native population is infected, a non-immune force with practically no malarial sickness. That magnificent result was only achieved by the great co-operation between the medical services and the staff, and that great co-operation they owed to Brigadier HAMILTON FAIRLEY whose persuasiveness convinced the Army of the practical value of stebrin suppression.

Dr D G Davey: Mr President, I should like to pay tribute, on behalf of Dr CURD, Dr ROSE and myself to Brigadier FAIRLEY and his colleagues for the magnificent work carried out at Cairns. The sincerity of my tribute may be better appreciated if I tell you very briefly what the results mean to us as laboratory workers. The curse of any initial investigations made on the chemotherapy of malaria is that one is forced to work with species that are different from those causing human malaria. I think we know a great deal about our laboratory species, but nevertheless the jump we make from the laboratory to the treatment of the human case is a very big one indeed. It was bad enough when we were concerned solely with the chemotherapy of the parasites of the red cells, but during the past few years we have made it worse. Throughout the war it was impressed upon us that what was wanted was no mere substitute for mepecrine. We were to look further. We were to obtain a drug which would achieve causal prophylaxis and the radical cure of benign tertian malaria. As you know in our search for such a drug, we became disciples of the gospel preached by the late Colonel S P JAMES—it saddens me that he is not here tonight to hear the results—and we studied the chemotherapy of exoerythrocytic forms. In other words, we tried, as intensively as we were able, to kill a form of the parasite which had not, and has not yet, been seen in human malaria. Paludrine is the best result to date of our researches. We were excited about it because of its action on exoerythrocytic forms, and now whichever way I look at Brigadier FAIRLEY's results, I am forced to conclude that it is acting, also, on the undiscovered exoerythrocytic forms of human malaria. For showing us that our hypothesis concerning these forms is probably correct we shall be always grateful to the Cairns workers. But there is one thing that frightens me. It is clear that paludrine is not the

## DISCUSSION

end of our task in the chemotherapy of malaria. We can say that it appears to be a very good drug, and that it should be of great practical value, but it has not all the accomplishments we should like in an anti-malarial drug. Consequently, the research goes on, and because of the interpretation which we must give to Brigadier FAIRLEY's results, we hold that it is the chemotherapy of exoerythrocytic forms that will give us the answer to our problems. What frightens me is this. If we or some other laboratory should find an improvement on paludrine, what shall we do? We shall want causal prophylactic experiments, and curative experiments with cases whose history is completely documented. And Cairns has shut down. If we make no attempt to put something in its place I cannot help feeling we shall be guilty of the gravest folly. The foundations of experimental work in human malaria have been well laid at Cairns and surely they should be built upon?

Aside from the precision of the work and the clarity of the results from Cairns, and the great assistance which they give us in creating a composite picture of human malaria, there is another point which ought not to be overlooked. It may not be generally known that paludrine was only synthesized in November, 1944, and it was still in birds in January, 1945. Today, therefore, it is only about 18 months old, and I would say that we already know more about it than we knew about mepacrine at the commencement of the war, and mepacrine, then, was about 10 years old. The amazing speed with which paludrine has been developed is due, first, to the happy collaboration which we had with the workers of the Liverpool School of Tropical Medicine, but also, in great measure indeed, to the work at Cairns. It is possible that much human suffering has been relieved because war conditions allowed us to experiment with paludrine in human beings, I argue that much human suffering could be relieved in the future if means are found for the experiments to continue. We are not asking too much. In all the experiments at Cairns, as Brigadier FAIRLEY has said, almost a thousand volunteers were used and there was not one fatality either from drug treatment or from malaria.

One thing more. Last December I had the privilege of accompanying Brigadier FAIRLEY to Cairns. There is much that I could say about my visit, but I will confine myself to mentioning three things which struck me. First, the all-round competence of the staff at Cairns. Secondly, the enthusiasm which abounded amongst them. Thirdly, the intimacy that had been developed between them and Brigadier FAIRLEY, which made itself apparent in the way every experiment was thrashed out and discussed at length. I think all three things were a consequence of the fact that Brigadier FAIRLEY identified himself with Cairns. I emphasize that he achieved this intimacy with his field station in spite of the fact that his headquarters were at Melbourne, roughly 2,000 miles from Cairns. The moral I want to draw is this. If we go 2,000 miles from London we are well on our way to Tropical Africa. I do hope we see to it that we go all the way and set up field laboratories there. One thing I am

confident about. The work with paludrine has shown clearly how essential it is that laboratory workers should know the field problems, that the field workers should appreciate the laboratory problems, and that, between all workers, there should be happy and enthusiastic collaboration.

One request I should like to make to Brigadier FAIRLEY. It was clear from the charts we have seen that he experimented with a whole range of doses varying from 10 mg. up to 1 gramme. I know he deliberately searched for toxic signs. It would be of interest if he would tell us something about them.

Dr. C. C. Chesterman. I voice the appreciation of those of us who have known Brigadier FAIRLEY for many years, and have seen the quality of his work and his activities. He has shown the possibility of disarticulating this menacing triangle of man, mosquito and malaria. We have been warned that paludrine may not be the panacea we look for the true parasiticide, but we realize that man and the mosquito are but the victims in the triangle, and that the real villain is the parasite. If we can concentrate on destroying the parasite where it is easily found in the human being, we have a method of disarticulating the triangle. Brigadier FAIRLEY has shown that it may be possible to have real causal prophylaxis and real gametocyte control. We should be anxious to start trying this on native populations. One piece of evidence we must get hold of first of all. Nothing has been said about children so far. Children keep many diseases smouldering. They do not suffer so intensely as adults from some such as yellow fever. But in endemic areas where we have a spleen rate of 100 per cent., children pay a heavy price for their tolerance to malaria, and can we lower that price? Weekly doses of quinine decrease the death rate but prevent the normal growth of tolerance. Later in life there is an attack, the patient is given a heavy dose of quinine and develops blackwater fever. What would happen if the children were taken from birth and given small regular doses of paludrine? Would it do more harm than good? One might save life for the first 2 years but render the children more susceptible when they get out of control and become infected. Would one have hesitation about the possibility of creating a resistant strain by doing that? Conceivably one might create resistant strains of the gametocytes and if that were to occur one would be in a worse position than before. I am grateful that Dr. DAVER has been able to supply me with paludrine for experimental purposes in a mission in Africa, where it will be possible to follow for many years its effect on a number of children as compared with quinine and controls with no treatment at all. That evidence we hope to be able to report at some future date and it should be of considerable value.

Dr. F. Murgatroyd. It has been a very great pleasure and privilege to hear Brigadier FAIRLEY tonight, and this Society in particular and the whole of Medicine in general, owe him an outstanding debt for his work, not only

for its specific scientific value but also for its striking demonstration of how much may be accomplished when technical ability is backed up by energy, determination and adequate facilities. As the previous speaker has well emphasized, this is a most important lesson, the implications of which should be carried over into the future.

In his spoken address Brigadier FAIRLEY has obviously not been able to give many of the details of the tremendous field of research that he and his colleagues have covered, and I should like to ask him about two matters. Firstly, as there is evidence that pamaquin (plasmoquine), combined with quinine, diminishes the incidence of relapse in benign tertian malaria, and as relapses of this infection are such a problem in malaria as seen in this country at the present time, I should like to ask if Brigadier FAIRLEY obtained any evidence that combining pamaquin with paludrine has any beneficially additive effect or therapeutically synergic action in lowering the incidence of relapses of benign tertian malaria. Secondly, although probably blackwater fever in any case would be rare under the conditions experienced, it would be interesting to know whether, in fact, any instance of haemoglobinuria was observed when treating malignant tertian malaria with paludrine.

**Dr Hugh S Stannus** Apart from the very valuable work carried out at Cairns in regard to malarial prophylaxis and therapy by means of paludrine described to us by Brigadier HAMILTON FAIRLEY, he mentioned one point, upon which other speakers have not commented, but which to my mind is of great importance, inasmuch as the principle involved may be one of wide application, namely, the experimental evidence suggesting that paludrine acts by interfering with the normal nuclear division of the malarial parasite possibly by depriving the organism of some factor taking part in some essential enzymic reaction. In this connection it seems worth while recalling some of the recent work in cancer research and the inhibitory action of colchicine on mitotic activity.

**Dr J D King** One question I would like to ask Brigadier FAIRLEY. I was not too sure from his report what was the highest dosage régime used in Cairns with paludrine. Also at what level of dosage he first saw manifestations of toxic effects from the drug, and what the toxic effects were?

**Brigadier Hamilton Fairley** (in reply) The hour is late, and I doubt if all these important questions can be adequately answered in the time at my disposal.

Brigadier SINTON and Dr CHESTERMAN have raised the question of dosage of paludrine to be adopted for non-immunes on the one hand and semi-immunes on the other. In regard to size of dose and duration of treatment, M T malaria can be rapidly cured by moderate dosage over a short period. With B T malaria a small dosage over prolonged periods holds out the best prospect of

radical cure. For suppression and causal prophylaxis in non-immunes I would advise 100 mg. of paludrine twice weekly given at 3 or 4 days interval with this regimen paludrine is a true causal prophylactic for M.T. malaria and a suppressant and partial causal prophylactic for B.T. malaria. For therapeutic 100 mg. thrice daily for 10 days produces radical cure in approximately 99 per cent. of *P. falciparum* infections. In vivax malaria this treatment can be continued with benefit for 3 weeks but better results would follow a maintenance dose of 100 mg. twice weekly for 6 months or longer. A dosage of one tablet (100 mg.) weekly is adequate to suppress benign tertian malaria, but for radical cure 100 mg. twice weekly would appear preferable.

The observations at Cairns that (1) a single dose of one tablet (100 mg.) may produce clinical cure of an attack of either *P. vivax* or *P. falciparum* malaria (2) 50 to 100 mg. of paludrine given at any time from the 39th to 120th hour following exposure to infection is a causal prophylactic in M.T. malaria are of great practical import. They raise entirely new issues regarding the control and final eradication of malaria in indigenous native populations. Presumably in the presence of premunity the suppressive or curative dosage could be further reduced and possibly the time interval between doses increased. At all events it would appear probable that 100 mg. every week would act as a causal prophylactic for many falciparum infections, and produce temporary clinical cure of overt attacks. Whether this dosage would ever produce radical cure in natives with premunity has to be ascertained. The dosage in children also remains to be determined, but 10 to 25 mg. would appear to be an adequate range for all requirements—10 mg. for suppressive purposes and 25 mg. for therapy.

Dr CHESTERMAN raises the pertinent question of the danger of curing malaria infections with loss of premunity so exposing the population at a later date to the ravages of epidemic M.T. malaria. Before premunity is established the death rate from M.T. malaria in infants and young children is high, and there is reason to believe that a tablet of appropriate dosage once or twice weekly would greatly lower the infantile mortality provided reasonable continuity of treatment could be assured.

Where paludrine was given once or twice weekly in the older age groups and to adults in whom premunity had been established, it would be essential to ensure that the administrative control responsible for distribution of the drug in the first place should also be available to act promptly in the event of an epidemic supervening. As already pointed out bi weekly administration at 3 or 4 days interval should ensure complete causal prophylaxis the one-dose-a-week regimen would considerably reduce the number of infections and exert beneficial therapeutic effects through schizonticidal action. Premunity is undoubtedly bought at a great price there is a high infantile mortality while chronic malaria in the older age groups produces widespread ill health, anaemia, chronic splenomegaly, cachexia and lowered resistance to secondary infection. The economic loss to the community in man-hours and in working efficiency is tremendous. In my opinion, the time has now come for large controlled field

experiments in hyperendemic areas in the tropics to ascertain exactly what chemotherapeutic control with paludrine can accomplish in the native villages and during epidemics. With a very small dosage given at weekly or longer intervals it may be possible gradually to acquire a degree of immunity without either the high death-rate or the concomitant ill effects of chronic malaria. If this proves impossible or impracticable the question arises whether in many parts of the tropics complete causal prophylaxis and radical cure of overt malaria should not be frankly aimed at, especially as paludrine will enormously increase our chemotherapeutic control of epidemics. Furthermore, in centres like South-East Europe, where there is a limited malaria season, chemotherapeutic control would appear to hold out assured prospects of success, for the only carry-over into the non-malaria season should be persisting vivax infections. Only carefully controlled and extensive field investigations will finally answer these questions and on such information will be based our future policy on the chemotherapeutic control of malaria in indigenous populations in malarious countries.

Dr DAVEY and other speakers asked about toxic effects. No significant toxic effects have been noted with the usual routine dosage of 300 mg daily or with a suppressive regimen of 100 or 200 mg weekly, or with 100 mg daily. In a few cases toxic effects have been observed when doses of 1.0 gramme daily have been given for the treatment of overt attacks. Such large doses occasionally caused vomiting and abdominal discomfort and in one or two cases oesophageal spasm was a possible complication. Evidence of renal irritation was occasionally indicated by the presence of sheets of epithelial cells and red blood corpuscles in the urine. In two cases gross haematuria occurred, in one of these 1.0 gramme was given in a single dose instead of in divided doses. An increase in myelocytes up to 10 per cent of the total leucocytes was observed on the 6th to 9th day in certain individuals with overt malaria while receiving 1.0 gramme of the drug daily, no decrease in the total leucocytes occurred. With the same dosage only a slight increase in myelocytes (1 per cent) was noted in normal volunteers who had not been exposed to malaria infection.

Brigadier SYTON has pointed out that one of the remarkable features of paludrine is the great latitude allowed between the optimal dose and the toxic dose. Large doses such as 1.0 gramme daily do not appear necessary, but if they are used trouble will be minimized by dividing the dosage and administering it after meals with a copious drink of water. In overt MIT malaria vomiting appears to be more troublesome when large doses are given and when fever is present, some reduction in dosage may then be desirable for the first 2 days.

Brigadier SYTON asks "What are the advantages of paludrine over atabrin and other drugs, especially as a suppressor?" In my opinion, paludrine will replace other known suppressive drugs for the following reasons: (1) the small dosage in which it is effective, and the great difference between the therapeutic and the toxic dose, (2) lack of toxic features in the dosage used, (3) lack of discoloration of the skin, (4) one tablet (100

mg.) twice weekly is a complete causal prophylactic for M.T. malaria and a partial causal prophylactic for B.T. malaria. (5) a single dose of even 100 mg. has proved an effective schizonticide resulting in clinical cure when administered during an overt attack of vivax or falciparum malaria. One small bottle of 104 tablets (100 mg.) would afford a year's supply for non-immunes or if given once weekly in the villages it would constitute 2 years' supply. This would enable easy distribution and presumably low cost.

Dr MURGATROYD asked whether there was any added advantage in giving paludrine with plasmoquine. Though no evidence of synergism has been obtained, both these drugs act on the pre-erythrocytic forms, while in vivax infections in short courses of treatment there is evidence that paludrine and plasmoquine are much more efficacious than paludrine alone. The course adopted has been 300 mg. of paludrine and 30 mg. of plasmoquine daily for 10 days. With this dosage no enhanced toxic effects of plasmoquine have been observed in Australia where many hundreds of patients have already been treated. To date the results obtained in vivax malaria with combined quinine (30 grains daily) and plasmoquine (30 mg. daily) treatment have been similar to those with paludrine and plasmoquine, except that in those cases which have relapsed the interval of freedom from attack has been approximately twice as long in the paludrine plasmoquine as in the quinine-plasmoquine series.

Dr STANUŠ referred to the experimental evidence that the chief action of the drug appeared to be in preventing nuclear division. Captain BLACK has clearly shown this action in cultures of *P. falciparum*, and Major MACKERRAS and Lieutenant ESCOLZ in careful and detailed studies on slides of *P. vivax* collected at 2 to 4-hourly intervals throughout the 48-hour cycle from vivax patients receiving paludrine. Similar action in inhibiting development of the oöcyt was noted by Major MACKERRAS and Lieutenant ESCOLZ in mosquitoes fed on falciparum and vivax gametocyte carriers taking paludrine. Indirect evidence of inhibition of schizogony in the "primary tissue" or pre-erythrocytic stage of the parasite was obtained in both vivax and falciparum infections by subinoculation technique. Nothing is known concerning the mechanism by which nuclear division is prevented. It occurred to me, however that if enzyme or co-enzyme systems were involved in the process of nuclear division, interference along the lines known to occur with other drugs might possibly constitute the explanation and provide an avenue worthy of exploration.

The President. I am sure you will want me, on your behalf, to thank Brigadier FAIRLEY for the most interesting and instructive evening he has been the means of giving us, and to congratulate him and the members of his staff at Cairns for bringing to such a successful issue the laborious and painstaking investigations on which they have been engaged. It is impossible to over estimate the value of the work that has been done.

## COMMUNICATIONS

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### THE EFFECT OF ANTI-MALARIAL DRUGS ON *PLASMODIUM FALCIPARUM* (NEW GUINEA STRAINS) DEVELOPING *IN VITRO*\*

BY

ROBERT H BLACK, Captam, A A M C

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#### INTRODUCTION

The mode of action of anti-malarial drugs has long been a matter for debate FIELD (1938) concluded that it was not known definitely whether the curative effect of quinine is by direct action on the parasite itself or indirect through mobilization of the defences of the host

MÜHLENS and KIRCHBAUM (1924) added quinine to blood containing parasites (*Plasmodium vivax*) and allowed it to act for 5 hours The blood still remained infective CHOPRA *et al* (1936) were able to kill malarial parasites *in vitro* with concentrations of quinine, atebirin and plasmoquine higher than are obtainable in the human body

Studies have been made on the depression of respiration of parasites when anti-malarial drugs are added *in vitro* to blood containing parasites FULTON and CHRISTOPHERS (1938) found that quinine and atebirin caused inhibition of respiration of *P knowlesi* Further work on the same line by COGGESHALL and MAIER (1941) showed that the results of these experiments did not always agree with the observed therapeutic effect

HEWITT and RICHARDSON (1943) added drugs as powder and in solution to blood infected with *P lophurae* and incubated at 6° C They found that typical degenerative changes occurred in the parasites when plasmoquine was used These changes were similar to those seen in the parasite when infected ducks were treated with plasmoquine They failed to produce degenerative changes with quinine and atebirin

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THOMSON and McLELLAN (1912) and DUDGEON and CLARKE (1917), discussing their cultivation of malaria parasites, thought that quinine in the patients sera had adversely influenced the development of the parasites.

The work of BOCK and OSTERLIN (1938-39) with photofluoroscopic examination of the parasites in monkeys treated with atabrin indicates that the drug has some direct action on malaria parasites.

It appears that there has been no systematic investigation of the effect of anti malarial drugs on human malaria parasites developing *in vitro*. The object of this present study was to determine if there were indeed any effect and, if so, at which stage in the schizogonous cycle it occurred.

Blood from volunteers at L.H.Q. Medical Research Unit, Cairns, Australia, was used for these studies.

### METHOD

The parasites (*P. falciparum*, New Guinea strains) were cultivated as described in a previous article (1945). Blood withdrawn from the patient was defibrinated by stirring and, after centrifuging, the red cells were taken from beneath the leucocyte layer.

A flat bottomed test tube (7.5 x 1.25 cm.) was used as a culture tube. It contained a layer of infected cells under a column of serum to which 0.1 c.c. of 50 per cent. glucose had been added. The tube was graduated at 8 c.c. giving a column of 5 cm. of serum.

Three large drops of packed red cells were added by dropping from just above the surface of the serum, mixed by twisting the tube, and then allowed to settle. It was thought that this would result in a better admixture with the serum than would occur if the red cells were run in as a layer. Red cells of group O-4 were used so that they would not be agglutinated or lysed by serum from donors belonging to other blood groups. Cultures were incubated aerobically at 37° C.

The schizogonous cycle in cultures of malaria parasites was fully described and illustrated by THOMSON and McLELLAN (1912) but a brief description of the behaviour of control cultures of *P. falciparum* cultivated and studied in this investigation will give a clearer picture of the results obtained when these parasites are grown in the presence of various drugs.

Small rings advance with increase of cytoplasm and become large rings. Parasites are then seen with irregularity of the cytoplasm (amoeboid forms). At this stage some pigment appears. Next there is a compaction of the cytoplasm and also of the pigment (preschizont). The chromatin material increases and becomes scattered throughout the parasite without definite segmentation (referred to as early schizonts in this article) and then divides into discrete particles which vary greatly in number. The cytoplasm condenses around the chromatin particles and a fully developed schizont is formed containing up to thirty merozoites. Rupture of the schizont with release of the merozoites and

the entry of these into other red cells to form young rings can readily be seen. All parasites do not complete the cycle for a proportion develop into atypical degenerate forms.

Usually cultures were made with a moderately heavy infection of the order of 100,000 parasites per cubic millimetre in the blood used so that many parasites could be examined in a thin film preparation. It was observed that the times taken for full development of schizonts varied according to the age of the trophozoites at the commencement of cultivation. In some cultures small rings of the second generation of parasites were not seen before 72 hours.

To estimate the effect of a particular drug on the developing parasites serum from a healthy donor to whom this drug was being administered was used for the culture. This method differs from that of HEWITT and RICHARDSON (1943), who added drugs directly to red cells containing parasites and incubated their preparations at a much lower temperature to prevent haemolysis. In this investigation anti-malarial drugs were given orally or by intramuscular injection and the sera from the recipients of the drug were used in the culture preparations to observe the action of such sera on the parasites in culture.

The drugs investigated in this manner were paludrine (M4888), M4430, atebirin, sontochin (SN6911), resochin (SN7618), plasmoquine, quinine, sulphadiazine, and sulphamerazine. A qualitative result regarding action *in vitro* was sought rather than the determination of the exact serum concentration at which the drug was effective. In most cases this could be secured with the dosage usually administered in the treatment of a patient ill with malaria.

Cultures containing serum from healthy donors treated with anti-malarial drugs were compared with parallel control cultures in which the serum contained no drug. Samples were taken from the cultures at sufficiently frequent intervals (usually every 4 to 6 hours) to allow examination of morphology at all stages of development. The thin films made from the cultures were stained with Leishman. Differential counts of parasites were made on each sample.

Estimations of the concentration of drug in the serum used in these cultures were carried out for atebirin by the Brodie and Udenfriend method, for quinine by the Brodie and Udenfriend method and for the sulphonamides by Fantl's modification of Werner's method.

It was necessary to use several strains of parasites in this investigation.

## RESULTS

### *Control Cultures*

The behaviour of these cultures has already been described. Two types of control were used for each experiment. Serum from the patient providing the parasites was used for the first and serum from a healthy donor taking no drug for the second culture. Eighteen control cultures were followed

through schizogony to re-entry of red cells by merozoites. There was no apparent difference in the behaviour of the parasite in the two types of control cultures.

#### PALUDRINE (M4888).

Thirteen cultures containing varying amounts of paludrine showed a very characteristic behaviour. The developmental cycle proceeded in parallel with control cultures until the early stages of schizogony were reached. Then the parasites in cultures containing paludrine ceased to develop. The scattered chromatin material did not divide into definite segments but became somewhat granular. The parasite and its containing red cell became swollen and vacuoles appeared in the cytoplasm. The figure illustrates the formation of the typical forms seen in these cultures. Thus when a control culture showed completion of the cycle with re-entry of red cells by merozoites a culture containing paludrine showed arrest of development of the parasites at the stage of early schizonts and these forms were undergoing degenerative changes. These forms are essentially similar to those seen in cases of vivax malaria (New Guinea strains) treated with paludrine which continue to circulate in the peripheral blood for several days after the commencement of therapy and undergo no further development. Degenerate early schizonts were seen in the red cells of the marrow when sternal puncture was performed on a patient suffering from overt falciparum malaria 52 hours after the commencement of paludrine therapy and, on one occasion, have been seen in small numbers in the circulation after treatment of a heavy falciparum infection with paludrine.

No method was available for the estimation of the paludrine concentration in the sera of these cultures. The thirteen sera used were obtained from donors to whom paludrine was administered as follows: four on a course of 1.0 gramme per day, one 2½ hours after 0.5 gramme, one 4 hours after 0.3 gramme, one on a course of 0.3 gramme per day, three 4 hours after 0.3 gramme, one 3 hours after 0.1 gramme, one 2 hours after 0.1 gramme and one 1 hour after 0.1 gramme. Four different strains of parasites were used in these cultures.

An experiment was performed to obtain some idea of the absorption rate of paludrine. A fasting donor was bled at intervals after the administration of 0.1 gramme of paludrine and the sera obtained used in a series of cultures. The parasites completed the full cycle in the serum of the donor collected prior to drug administration. Cultures in the sera from specimens collected 1 hour, 2 hours and 4 hours after drug administration resulted in the typical changes which have been described. A specimen collected half an hour after the drug was swallowed showed only partial arrest—some parasites went through schizogony while others were arrested at the stage of early schizonts.

Another experiment showed that *P. falciparum* trophozoites (ring forms) which had been exposed for about 3 hours to paludrine in a treated patient developed normally when cultivated in normal serum. They were arrested



FIG 1—Normal dividing schizont as seen in control culture of *P. falciparum*

FIG 2—Early schizont from a culture of *P. falciparum* made in serum from a donor to whom paludrine had been given. Note early vacuolation

FIGS 3-5—More vacuolation is seen, then swelling and disappearance of the cytoplasm. Failure of chromatin division

FIG 6—Disintegration of the chromatin



at the early schizont stage when grown in the serum of the patient who had had 0.1 gramme of paludrine 4 hours before the commencement of the cultures. This experiment was performed three times with the same result.

### M4430

Arrest of development of parasites similar to that seen with paludrine was obtained when they were cultivated in serum from a donor taking a course of M4430, 0.4 gramme per day.

### ATEBRIN

Parasites were grown in sera collected from donors who had been given varying amounts of atebirin di-hydrochloride orally or by intramuscular injection. Eight test cultures were made using three different strains.

With serum atebirin levels up to 120  $\gamma$  per litre there was no effect on the development of the parasites but with higher concentrations such as 190  $\gamma$  per litre and 300  $\gamma$  per litre the parasites did not develop beyond the amoeboid stage. The amoeboid forms degenerated with loss of definition in the staining of the cytoplasm and eventually underwent disintegration. Many ring forms degenerated without undergoing any development. The red cells containing these parasite fragments ruptured and became ghost cells.

### SONTOCHIN (SN6911—bi-sulphate)

Parasites were grown in serum from a donor to whom sontochin had been given in doses sufficient to secure a calculated plasma concentration of the order of 300  $\gamma$  per litre. Changes were seen in the parasites similar to those observed when the sera containing higher concentrations of atebirin were used as culture media. A few amoeboid forms appeared and then these and the ring forms degenerated leaving chromatin fragments in the red cells.

### RESOCHIN (SN7618—di-phosphate)

Resochin 0.5 gramme was administered daily to two donors, who were bled 2 hours after the morning dose of 0.3 gramme on the 3rd day. This gave a calculated plasma concentration of 200 to 300  $\gamma$  per litre.

Parasites grown in sera from these donors showed changes similar to those using sontochin. Very few amoeboid forms developed. These and the ring forms showed disintegration of the cytoplasm, leaving only chromatin material in the containing red cells.

### QUININE

Six cultures were made in sera from donors taking quinine sulphate mixture orally. The parasites were obtained from four patients with falciparum malaria of different strains. The serum concentrations ranged between 6.0 and 8.8 mg

of quinine base per litre. With three strains the parasites did not progress further than the preschizont stage and the forms then present—ring, amoeboid and a few preschizonts—underwent degeneration and fragmentation with the formation of ghost cells from the containing red cells. Parasites of the fourth strain did undergo schizogony with re-entry of red cells by merozoites but the cycle was retarded and the number of schizonts formed was less than in the control cultures.

#### PLASMOQUINE.

A donor was given 0.02 gramme of plasmoquine (expressed as base) 2 hourly for four doses. He was then bled 3 hours after the last dose. Two cultures with different strains were set up using the serum from this donor. In one there was no development of the parasites beyond the formation of a few amoeboid forms. In the other the cycle was completed after a considerable delay in the early stages.

#### SULPHONAMIDES.

Two cultures were made using serum containing sulphadiazine 13.7 mg. per 100 ml. The parasites developed normally up to the stage of division of the chromatin into definite segments. Then the great majority of these schizonts degenerated. The cytoplasm developed vacuoles, and then lost its blue staining with Leishman. The chromatin segments became irregular and eventually ghosts of red cells were seen containing parasite remnants only. Further observation of these cultures revealed the appearance of a very few rings of the second generation—thus all schizonts in the culture had not been destroyed.

In a culture made with serum containing sulphamerazine 16.9 mg. per 100 ml. the parasites were slow in developing compared with the controls. Here also were seen degenerate schizonts and the development of only a few rings of the second generation.

#### DISCUSSION

It appears that in these cultures the drugs which were used in the form in which they circulate in the blood had a direct effect on the developing trophozoites of *P. falciparum*. They caused either a delay in the development of parasites or arrest of development and subsequent degeneration.

The action of atabrin, sontochin, resochin and quinine was on the early stages of the schizogonous cycle, whereas that of paludrine and M4430 was not exerted until later—at the stage of division of the chromatin of the schizont while the sulphonamides tested acted on the divided schizont. It is realized that quinine, atabrin, sontochin and resochin may have a lethal action on the later stages of the cycle. In the cultivation method here described they either destroy the earlier forms or allow the completion of schizogony. Thus in culture the schizont is not markedly more susceptible to the drug than are the earlier forms of the parasite. It has been suggested by FIELD (1938) that

degeneration of the red cell containing the segmenting parasite permits entrance of anti-malarial drugs but from the results obtained with these cultures it appears that the red cell allows entrance of these drugs when it contains early forms of the parasite.

In the treatment of a case of overt malaria the drug is administered throughout the whole of the schizogonous cycle and has ample opportunity to act on all stages of development of the parasite. This circumstance is approximated when a culture of living parasites is exposed to a drug although the drug is at a more even concentration than it is in the body.

It has already been mentioned that the drug is present in these cultures in the form in which it circulates in the body. In the case of atebryn given by intramuscular injection the serum for the cultures was obtained from blood taken only 15 minutes after the injection and yet it was as effective as serum with similar concentrations of atebryn which had been ingested. Thus, if atebryn must be in a combined or altered form before it can act on parasites, alimentation is not necessary for this modification of the drug and the change occurs within 15 minutes of its intramuscular injection.

No definite evidence was found that drugs present in a concentration which allowed schizogony to occur prevented merozoites from invading red cells. Whenever mature schizonts occurred in a culture followed by the liberation of merozoites, rings of the second generation were always seen.

Specific immune properties of the sera used in the culture were excluded as an influence detrimental to the parasite since the parasites were taken from patients with a primary attack and the drugs were given to donors who had not had falciparum malaria.

Mobilization of the defences of the body has been suggested as the method of action of anti-malarial drugs. If this is so it must be of a non-specific nature and might be said to be (at least as far as this culture work is concerned) the absorption of the drug and its presence in the blood in its circulating form.

Leucocytes play no part in the disposal of parasites in these cultures as they are removed before cultivation is begun. Parasites *in vivo* may, however, be affected by anti-malarial drugs in a manner which makes their phagocytosis by leucocytes more readily accomplished. The fragmentation of degenerated parasites, and particularly the rupture of the containing red cells to form ghost cells, is notable. If this occurs in patients receiving treatment for malaria, then the parasite fragments and the damaged red cells would be readily removed from the circulation by cells of the reticulo-endothelial system.

Although most of the parasites undergo schizogony in a control culture containing no drug, the second generation does not progress so readily. Thus the method of cultivation described does not provide ideal conditions for the development of the parasites. Parasites growing under this handicap might readily be destroyed by any adverse condition, including the presence of such drugs as were used, and the results obtained might not therefore be comparable



to those found *in vivo*. Two of the findings point against this. Firstly the sites of action of some of the drugs in the malarial cycle are different—as with atebirin and paludrine, and, secondly the characteristic degenerating only schizonts are found with paludrine *in vitro* and *in vivo*. The method then may be accepted as one which will give results which may be used in the explanation of the action of these drugs on the trophozoites when overt malaria is treated *in vivo*.

Thus the lethal effect of anti-malarial drugs, in the serum used for cultivation, appears to be due mainly to their being present in sufficient concentration to exert a direct influence on the developing parasites. This probably is also the case in the treatment of an overt attack of malaria. Drugs used for suppression of malaria are present in the blood at lower concentrations but, in this circumstance, other factors are involved, such as (1) the prolonged exposure of the parasite to the drug (2) the presence of active leucocytes (3) the reticulo-endothelial system and (4) the development of immunity.

#### SUMMARY

- 1 A method is described which has been used to study the effects of drugs on trophozoites of *P. falciparum* (New Guinea strains) growing *in vitro*.
- 2 The effects of sera containing various anti malarial drugs—paludrine, atebirin, sontochin (SN6911), M4430 resochin (SN7618) plasmoquine, quinine sulphadiazine and sulphamerazine—on these parasites are described.
- 3 Atebrin, sontochin, resochin and quinine were observed to cause arrest of development and degeneration of ring and amoeboid forms.
- 4 Paludrine and M4430 exerted their lethal effect on the early schizonts.
- 5 The sulphonamides caused degeneration of divided schizonts.
- 6 Plasmoquine had inconstant or only minor effects.
- 7 The degenerating forms seen when paludrine is used *in vitro* with *P. falciparum* and *in vivo* with *P. vivax* are similar.
- 8 The mode of action of anti malarial drugs is discussed.

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## CONCERNING EXOERYTHROCYTIC FORMS AND THE EVIDENCE FOR THEIR EXISTENCE IN HUMAN MALARIA

By

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In the following paper I have attempted to make a brief survey of human malaria from the viewpoint of a laboratory worker desiring to discover better drugs for its therapy and control. Drugs which are particularly required are those for causal prophylaxis,\* and for the radical cure of *Plasmodium vivax* infections. What special accomplishment such drugs should have is a matter for debate, and in searching for them one is compelled to theorize. Thus, do mepacrine and quinine fail to cure benign tertian malaria because their distribution in the body is at fault, or is there a form of the parasite, such as an exoerythrocytic form, which is refractory to them? Although the questions cannot be answered definitely for human malaria it is clear that the causal prophylaxis and radical cure of vivax malaria is intimately concerned with the chemotherapy of exoerythrocytic forms. This fact determined the approach followed in these laboratories in attempts to discover new drugs for human malaria (CURD, DAVY and ROSE, 1945; DAVY, 1946) and because some success has attended our efforts it seems an opportune time to review together

\* I define a causal prophylactic drug as one which prevents parasites appearing in the red blood cells, it may act on the sporozoites or on a stage existing between the sporozoite and the blood parasites.

the respective pictures presented by the chemotherapy of avian and human malaras. Our information concerning the chemotherapy of human malaria, particularly that derived from experimental work, is much greater for infections with *P. vivax* and *P. falciparum* than it is for infections with *P. malariae*. Consequently this discussion is dominated by the first two species.

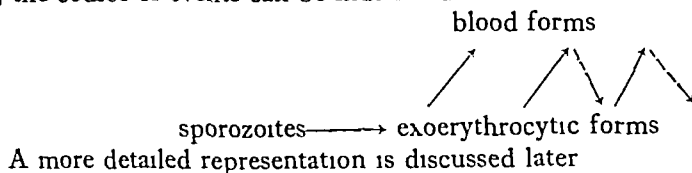
The purpose of the review is to encourage still further the spirit of mutual assistance between workers with experimental infections and workers with human malaria which has proved so fruitful during the war years. It would be difficult, for example, to exaggerate the value to the laboratory worker of experiments such as those done at the Australian Army Research Station at Cairns (FAIRLEY 1945-1946), and although it is perhaps too optimistic to suppose that work on the scale seen at Cairns can be arranged in times of peace, yet much important information remains to be obtained. It can be obtained if the problems are kept in view.

### EXOERYTHROCYTIC FORMS IN AVIAN MALARIA.

The term exoerythrocytic was first used by JAMES and TATE (1937) to describe certain forms of *P. gallinaceum* which they discovered in chicks. These forms were found in cells of the reticulo-endothelial system and in endothelial cells in various organs of the body. They differed from the parasites of the red blood corpuscles in two main ways. First, because haemoglobin had never been used in their metabolic processes, pigment (haematin) was absent from them. Secondly their size seemed to be limited only by the type of cell in which they developed—those in the endothelial cells of the brain capillaries for example, were relatively enormous. Similar forms had been described in infections of canaries with *P. relictum* (RAFFAELLI, 1936a) and with *P. cathemerium* (KIKUTH and MUDROW 1937).

Since the publication of the papers of JAMES and TATE (1937-1938) a great deal of work has been done searching for these forms and experimenting with them in different species of avian malaria—much of it is reviewed by PORTER and HUFF (1940). The "gallinaceum type" has been described in infections of *P. cathemerium*, *P. circumflexum*, and *P. relictum* in canaries, and *P. lophurae* in turkeys and pheasants. They are found most easily by taking impression smears of the tissues in sporozoite-induced infections at the time when parasites are first found easily in blood smears—earlier than this too few have developed for the task to be less than difficult, and later if the host survives, the numbers again diminish. Their exact relationship to the sporozoites could only be conjectured until HUFF and COULSTON (1944) described for *P. gallinaceum* the complete sequence of events between the injection of sporozoites and the appearance of parasites in the red cells. They showed, for this species at least, that the sporozoites penetrate the cells of the reticulo-endothelial system within half an hour of their introduction into the host, and therefore, in the broad sense of the term, become exoerythrocytic forms. Afterwards, for the

duration of the infection, an unbroken succession of such forms occurs. Sooner or later, they throw off blood forms (for times see HUFF and COULSTON, 1944, COULSTON, HUFF and CANTRELL, 1945, DAVEY, 1946). At first, only cells of the reticulo-endothelial system are parasitized, later ordinary endothelial cells are also invaded (PORTER, 1942, HUFF and COULSTON, 1944). In a general way, the course of events can be illustrated as follows —



### THE EVIDENCE THAT EXOERYTHROCYTIC FORMS EXIST IN HUMAN MALARIA

Following the discovery of exoerythrocytic forms in avian malaria reports have appeared from time to time claiming the discovery of similar forms in human malaria (*e g*, RAFFAELE, 1937a, CASINI, 1939, BRUG, 1941). Unfortunately, the supposed parasites which have been described are very few in number, and in every instance it is possible to suggest an alternative interpretation of the observed structure. It must be admitted, therefore, that no indisputable microscopical demonstration of the presence of exoerythrocytic forms in human malaria has yet been made, and the evidence for their existence is indirect. It emanates from two main sources, the results of subinoculation experiments and the effects of drugs.

#### (a) THE EVIDENCE FROM SUBINOCULATION EXPERIMENTS

If sporozoites penetrated red blood corpuscles directly, as described by SCHAUDINN (1902), one would expect that the blood of the vertebrate host should become immediately parasitized and should remain so. That this does not hold has been shown in the case of both *P. falciparum* and *P. vivax* (*e g*, BOYD and STRATMAN-THOMAS, 1934, BOYD and MATTHEWS, 1939, RAFFAELE, 1937b, CIUCA *et al*, 1937, FAIRLEY, 1945), and in the case of various species of avian malaria (*e g*, RAFFAELE, 1936b, WARREN and COGGESHALL, 1937, COULSTON, CANTRELL and HUFF, 1945, DAVEY, 1946). Instead, there occurs a very transient period of infectivity followed by a prolonged period of non-infectivity—the “negative blood phase”. It is true that a negative blood phase also follows the injection of organisms such as bacteria and viruses, which are removed from the blood stream by the reticulo-endothelial system, but the phenomenon has a special significance in malaria because it is the red blood corpuscles themselves which later come to be parasitized.

Some of the earlier work on the negative blood phase in human malaria may be criticized on the score that the experiments were meanly conceived

Few subjects were used and only small quantities of blood were subinoculated. But such criticisms cannot be aimed at the work of FAIRLEY and his collaborators. Using volunteers they have shown that, following the bites of mosquitoes heavily infected with sporozoites of *P. falciparum* or *P. vivax* subinoculation yields positive results within the next half hour and then consistently negative results until the 7th day of infection with *P. falciparum* and the 9th day with *P. vivax*. In these experiments at least 200 ml. of blood, sometimes 500 ml., have been transfused directly from one volunteer to another and the precision with which the results have been confirmed, time after time, is striking. One must conclude that the sporozoites of *P. vivax* and *P. falciparum* disappear from the circulating blood, and parasites do not reappear there for several days afterwards.

The parallelism between the results in human malaria and avian malaria is exact apart from the details of time, and it can be taken as generally true that, wherever else the sporozoites develop they do not develop in the red blood corpuscles. In the broad sense of the term, then, their development must be exoerythrocytic. The details of this development, as was remarked above, have been worked out only for *P. gallinaceum* in which species it has been shown to take place in the reticulo-endothelial system. The experiments with *P. gallinaceum* have a negative as well as a positive importance. The clear demonstration that the sporozoites of this species develop in the reticulo-endothelial system is, of course, strongly suggestive when explanations are sought for results such as those of FAIRLEY but the difficulties of making the demonstration should be borne in mind when deplored the failure to do so for other species. HUFF and COULSTON injected the salivary glands from as many as 300 mosquitoes into a single chick, and even then found it necessary to localize the development of the sporozoites in a restricted area of wingskin, before they were able to describe a complete series of stages. In my own experiments (DAVEY 1946) following the intravenous inoculation of the contents of fifty mosquitoes into a 6-day-old chick, I found the greatest difficulty in finding even isolated examples of developmental stages. Work on this scale in chicks is roughly comparable to injecting the contents of 50,000 mosquitoes into a human being, and clearly therefore, the failure so far to demonstrate the exoerythrocytic developmental stages of the sporozoites in human malaria is not evidence that they do not exist.

#### (6) THE EVIDENCE FROM THERAPEUTIC EXPERIMENTS.

##### 1 Causal Prophylactic Experiments

In their experiments on the treatment of general paretics with malaria YORKE and MACFIE (1924) showed a striking difference in the protective value of quinine according to whether it was given during the incubation period to patients infected by the inoculation of parasitized blood or by the bite of

mosquitoes. It cured the former but did nothing more than slightly delay the onset of the clinical attack in the latter.

These results were confirmed and extended by JAMES, NICOL and SHUTE (JAMES, 1931a). The same workers, in 1931, showed that pamaquin (plasmochin, plasmoquine) produced results quite different from quinine. Administered on the first 6 days of the infection, admittedly in toxic doses (0.02 gramme t.i.d.), it gave complete protection against malignant tertian malaria and caused an astonishing lengthening of the incubation period of benign tertian malaria. Some of the volunteers infected with *P. vivax* did not exhibit clinical symptoms until 7 to 9 months after infection, and JAMES can be forgiven for supposing, at first, that pamaquin had completely protected them. The result, in any case, was exciting, and was all the more remarkable because the action of pamaquin against the parasites of the red cells was known to be comparatively poor. It, together with the quinine results, led JAMES to announce his belief (1931b) that sporozoites did not penetrate red cells directly, but developed elsewhere in the body, probably in the cells of the reticulo-endothelial system. He thought that the pamaquin actually killed the sporozoites, but it is not clear from his writings whether he thought the action occurred in the blood stream or the reticulo-endothelial cells. In conversation he has told me that, in those early days in the investigation, with no word to describe separately the hypothetical stage in the life-history which he had postulated, he used the word sporozoite in a very general sense to describe the parasite before it entered the red cell.

The findings of JAMES concerning the action of pamaquin have been amply confirmed during the last war both in Australia and in the U.S.A., and it can be taken as definite that, in causal prophylactic experiments, pamaquin possesses properties not possessed by quinine, mepacrine (atebrin, atabrine, quinacrine), sontoquin or S.N.7618 (resochin, chloroquine)\*.

The next causal prophylactic results to be obtained were those of SINTON, HUTTON and SHUTE (1939), who showed that prosectasine, one of the early sulphonamides, given in very high doses on the day before and the day of infection, gave complete protection against malignant tertian malaria. It was hoped that newer sulphonamides such as sulphadiazine, sulphamezathine and sulphamerazine, would have been an improvement on prosectasine, but work in the U.S.A.†, and in Australia at Cairns (FAIRLEY, 1945), showed that the protection conferred by them at practicable doses (0.5 to 1.0 gramme per day) was incomplete against *P. vivax* and, although good against *P. falciparum*, was insufficient to prevent parasites from entering the circulating blood.

Throughout the war an intensive search for a causal prophylactic drug went on in various laboratories in this country and in the U.S.A. One of the consequences of this search, for which results are available, is paludrine.

\* Reports of Brigadier N. H. FAIRLEY.

† Communications from the Board for the Co-ordination of Malarial Studies, U.S.A.

FAIRLEY and his colleagues (FAIRLEY 1946) have shown that this substance has a striking causal prophylactic action against *P. falciparum* and at least a partial action against *P. vivax*. In infections with *P. falciparum* parasites are prevented from entering the blood stream by doses as low as 25 mg. per day and a dose of 100 mg. taken on each of the first 3 days after infection, is sufficient to give complete protection. A dose of 100 mg. once daily also prevents *P. vivax* from entering the blood stream, but in this instance the action is incomplete and, following the cessation of treatment, overt attacks can be expected some months later. Other work at Cairns and the results of an experiment in this country done in collaboration between workers in our laboratories and the Liverpool School of Tropical Medicine, have shown that at higher doses paludrine may give complete protection against *P. vivax*. These other experiments are of more theoretical than practical significance.

The discovery of paludrine was a consequence of deliberately searching for a drug with an action on exoerythrocytic forms in avian malaria (CURT, DAVEY and ROSE, 1945; DAVEY 1946). Pamaquin, too, has an action on exoerythrocytic forms in avian malaria, and so have the sulphonamides (COXNER and COOPER, 1944; COOGESHALL, PORTER and LAIRD 1945; DAVEY 1946). We therefore have a group of drugs comprising pamaquin, prosectamine and paludrine, all of which are chemically distinct, all of which have an action in human malaria not possessed by quinine, mepacrine, sontochin, etc., and all of which have an action on exoerythrocytic forms in avian malaria not possessed by these others. Surely this is significant?

## 2. *The Radical Cure of Benign Tertian Malaria.*

For many years relapsing malaria has provided not only a target for chemotherapeutic investigations, but also a subject for debate. Two main explanations of why malaria is so difficult to cure have been advanced. The first suggests that a few parasites may be protected from the action of a drug by being sheltered in a sinus of the spleen, or in some other blood reservoir where the blood is temporarily taken out of circulation and where a drug does not penetrate. On the face of it this does seem a possibility but there is a very great obstacle, which seems to be generally overlooked, in the way of accepting it. Briefly blood induced *P. vivax* infections can be cured using drugs such as quinine and mepacrine, and sporozoite induced infections can not. If the difficulty of cure is simply a question of the distribution of parasitised red corpuscles why should there be this difference?

The second explanation suggests that exoerythrocytic forms are persistent in *P. vivax* infections (and possibly in *P. malariae* infections also). It is necessary to make distinction at least between *P. vivax* and *P. falciparum* because the work during the war has shown quite clearly that the radical cure of malignant tertian malaria is not a problem. It can be accomplished with virtual certainty using drugs such as mepacrine which, as far as one knows act only on blood

forms. Thus, the drugs which cure it do not necessarily prevent parasites from gaining access to the circulating blood when they are continually taken throughout the incubation period of the sporozoite-induced disease (FAIRLEY, 1945). In other words, if one presumes there are exoerythrocytic forms immediately following the sporozoites of *P. falciparum*, then drugs such as mepacrine are without action on them. If these forms were persistent mepacrine, of course, should not cure.

Persistent exoerythrocytic forms would provide an explanation of most of the problems associated with sporozoite-induced benign tertian malaria, and it is therefore important that at least indirect evidence can be brought forward to show that the explanation is not without foundation. The evidence can be summarized as follows.

1. There exist species of avian malaria (e.g., *P. cathemerium*, *P. relictum*, *P. gallinaceum*) with persistent exoerythrocytic forms which seem completely refractory to quinine, mepacrine, etc. The blood forms, on the other hand, are susceptible to such drugs in all these infections. It follows, therefore, that the infections can be controlled with them but not eradicated which, it will be admitted, is strikingly analogous with what obtains in benign tertian malaria.

2. The only known drug which exerts a true curative effect against *P. vivax* is pamaquin. It will be recalled that pamaquin has at least some action, albeit a poor one in some infections, against the exoerythrocytic forms of avian malaria.

3. A person with little or no immunity to malignant tertian malaria, if he is not cured, will relapse quickly, usually within 3 weeks of the cessation of the incomplete treatment\*. The period between attacks is never so long that it cannot be explained by supposing that the treatment was insufficient to eradicate the blood forms. In other words, the period is what one would expect the incubation period to be in a non-immune subject inoculated with a few blood parasites. This is not true of benign tertian malaria where many months may elapse between attacks.

Also, in causal prophylactic experiments with *P. falciparum*, if a subject does not exhibit clinical symptoms within about 4 weeks after the cessation of treatment he can be regarded as cured. This is clearly seen from the experiments done at Cairns (FAIRLEY, 1945, 1946). But in experiments with *P. vivax*, as the work with pamaquin and paludrine has shown, one must wait at least a year before drawing final conclusions. In searching for an explanation for this fact it is difficult to believe that blood forms are in existence and kept inhibited so long in subjects who have no acquired immunity towards them. Certainly, I do not believe that such a lengthy incubation period could be obtained in a non-immune subject by the inoculation of blood parasites, however few their number. On the other hand, the result could be explained by supposing

\* The relapse of malignant tertian malaria after 13 years reported by NAGLEY (1945) may be an example of premunition to a blood infection being lost.



that exoerythrocytic forms are present which, gradually increasing in number eventually release blood forms to bring about a clinical attack.

The evidence presented in the above paragraphs could be checked easily by subinoculation experiments. It is tantamount to saying that, if a person is not cured of malignant tertian malaria, he is carrying parasites in his blood, whereas this is not necessarily so in benign tertian malaria.

4 This is only negative evidence, if it is evidence at all, but it is worth bearing in mind the fact that the failure to demonstrate exoerythrocytic forms at any time of the infection in benign tertian malaria will not be of real significance until more work is done. It is often difficult to find them in canaries infected with *P. relictum* or *P. cathemerium* unless one chooses the optimum time to search, and in them one knows they are present, and one knows what to look for—the volume of tissues to be scanned is also much less. The importance of searching at the optimum time cannot be too strongly emphasized. It was remarked above that the exoerythrocytic forms in canaries are found most easily in sporozoite-induced infections at the time parasites are first found easily in the blood. Later even within a few days, their number may diminish considerably. Dr ANN BISHOP (personal communication) has made a special study of this point and her work clearly demonstrates its truth. It is probable that most of the attempts to find exoerythrocytic forms in human malaria have been made at a period in the infection which one would deem unsuitable in avian malaria.

#### PROBLEMS.

Apart from the overriding problem of making a precise demonstration of exoerythrocytic forms in human malaria, there are some others which are of interest and importance.

1 I know of no accomplishment possessed by pamaquin in avian malaria which is not possessed in at least equal degree by paludrine (DAVEY 1946). Also there is a remarkable parallelism between the results obtained with these two drugs in causal prophylactic experiments with benign tertian malaria (for the pamaquin results see JAMES, NICOL and SHUTE, 1931; JAMES, 1932 and the *League of Nations Health Committee Third Report on Malaria* and for the paludrine results see FAIRLEY 1946 and ADAMS, DAVEY and DAVEY in the press). Both drugs produce a very great lengthening of the incubation period if not actual cures. If one explains the results in terms of an action of the drugs on exoerythrocytic forms, and if one explains the action of pamaquin in reducing the relapse rate of benign tertian malaria in the same way then paludrine should also reduce it. If it does not, what is the explanation?

2 The parallelism between avian malarias, such as those due to *P. cathemerium* and *P. gallinaceum* and benign tertian malaria is not exact. Avian malaria which is blood induced is as difficult to cure as that induced by sporozoites,

again because exoerythrocytic forms develop. It seems as though they develop from the erythrocytic forms because COULSTON and MANWELL (1941) found them in infections of *P. circumflexum* which had been started by the injection of a single parasitized corpuscle, and I have tried, by differential centrifugation, to separate the parasitized red blood cells in *P. gallinaceum* infections from the other cellular constituents of the blood, but I have never failed to obtain exoerythrocytic forms by injecting the separated red cells. In benign tertian malaria, however, a blood-induced infection can be cured with quinine or mepacrine and a sporozoite-induced infection cannot be. If the difficulties associated with the latter cure are due to exoerythrocytic forms one can only conclude that they are not present in the blood-induced infections. From the viewpoint of the general hypothesis concerning exoerythrocytic forms it is unfortunate, therefore, that one of the supposed demonstrations of them in human malaria (BRUG, 1941) should have been in a blood-induced infection.

3 If exoerythrocytic forms arise only in lineal descent from the sporozoites of *P. vivax* the number of them in an infection should be proportional, in the first instance, to the number of sporozoites injected. It would be interesting, therefore, to investigate if there is a correlation between curative rate and degree of exposure to infection.

4 In avian malaria in which exoerythrocytic forms are coexistent with blood forms, it is a general rule that the infection persists for the duration of the host's life. It may be brought under control, but it is not usually eradicated. Treatment of the acute infection will frequently assist the host in attaining control.

Unfortunately, there is little accurate information concerning the longevity of human malarial infections. It was a common assumption by soldiers of the first world war that chills they were suffering in the thirties were due to malaria they had contracted during their war years, but undoubted clinical malaria, *i.e.*, parasites with fever, does not seem to have been recorded later than 2 to 3 years after infection\*. A greater accumulation of exact information would be important in the following way. The relapse rate quoted in various experiments with mepacrine or quinine is rarely 100 per cent. Following treatment with mepacrine of a primary attack caused by apparently virulent strains such as that used in the Cairns experiments the relapse rate may approach this figure, but in other centres it is recorded as perhaps 50 to 60 per cent, and sometimes is as low as 20 to 30 per cent. In general the relapse rate in subjects who have had one attack is less than that in subjects being treated for their first attack, and is less in those treated for a third than in those treated for a second, etc. A residue finally remain who may go on to have a dozen or more attacks. A question naturally arises concerning the true nature of the cures. Are they really radical cures or do they represent infection finally brought under control?

\*There are records of the parasites of *P. malariae* persisting for as long as 27 years (RUBENSTEIN *et al.*, 1945).



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## GROWTH OF PROTOZOA IN TISSUE CULTURE II—*PLASMODIUM RELICTUM*, EXOERYTHROCYTIC FORMS

BY

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This paper describes the growth of the exoerythrocytic forms of *Plasmodium relictum* in tissue culture by the technique already described for *P. gallinaceum* (HAWKING, 1945)

### TECHNIQUE

Canaries were inoculated intravenously each with the glands of about eight mosquitoes (*C. pipiens*) infected with the G strain of *P. relictum*. They were killed about the 8th day when trophozoites were beginning to appear in the erythrocytes. At this time, smears made from the spleen and liver contained only few exoerythrocytic forms (none to four in fifty fields). Small pieces of spleen were set up for tissue culture by the technique already described (HAWKING, 1945). The fluid medium in the early experiments contained 10 to 20 per cent of canary serum, but such serum was difficult to obtain and necessitated the sacrifice of many canaries. In later experiments it was found that the canary cells grew fairly well when they were embedded in a small amount of fowl plasma and were bathed in a fluid consisting of 20 per cent fowl serum, 20 per cent chick embryo extract, 60 per cent Tyrode plus penicillin to about 3 units per c.c. and enough phenol red to give a pink colour. The medium was changed about every 5 days. Growth of the canary cells in this medium was not quite as satisfactory as that of ordinary chicken tissue, the tissue tended to form an annular mass of elongated cells around a clear open space containing only the necrotic remains of flattened cells. Consequently the parasites were partly hidden by the thick cell mass in which they occurred, and they were less easy to study than was *P. gallinaceum*. For microscopical examination, one or more of the coverslips carrying the cultures was removed and fixed in Schaudinn's fixative. It was stained in Giemsa, differentiated in very dilute acetic acid for a few seconds, dehydrated by passing through appropriate mixtures of acetone and xylol, and mounted in a neutral mountant.

In many of the tissue cultures which have been made during this period, trouble was experienced due to the presence of small slender bacilli, growing in groups, often in the macrophages or in masses of material like mucoid. They did not appear until the 4th to 6th day, and they impaired the vitality of the culture without destroying it altogether. They could be removed by passing the embryo extract through a filter, so apparently they came from the chick embryos. Filtration through a Seitz filter impaired the growth-promoting properties of the extract, but filtration through a collodion one was less deleterious. The growth of these bacilli was not prevented by penicillin or by streptomycin about 5 units per c.c.

\* Grateful acknowledgments are due to Miss A. BISHOP, D.Sc., for valuable help, to Miss V. D. MARKHAM, Miss R. J. BERSON, and Miss V. PICKERTON for assistance with the experiments and the illustrations, to Mr F. V. WELSH, F.R.M.S., and Mr C. D. SUTTON for the photography, and to Dr S. A. WAKSMAN for the supply of streptomycin.

## EXPERIMENTAL RESULTS.

Successful cultures were made from the spleens of three canaries, and they were maintained up to 19 days, after which all the remaining colonies were removed for microscopical examination. Parasites were found both at the earliest time of examination (4th day) and at the latest (19th day). Food was removed from some of the flasks on the 5th and 9th days of cultivation and was injected into a canary which became infected 14 days after the first inoculation. In some of the cultures very vigorous growth of the parasites occurred, but as with *P. gallinaceum* it was unevenly distributed. In some places parasites were very numerous, in other adjacent areas they were completely absent. Apparently the merozoites do not spread very far from the spot where they have been liberated. Good growth of the parasites occurred in the presence of streptomycin, 5 units per c.c. When small pieces of liver were implanted in the birds instead of spleen, there was little or no growth of cells.

MORPHOLOGY OF *P. relictum* IN TISSUE CULTURE.

Many different forms of the plasmodium were seen in the cultures, and by selection of suitable types, the probable cycle of development can be illustrated. But since a single parasite cannot be followed throughout the whole course of the cycle, one cannot be certain whether some of the forms seen are really stages through which all parasites pass, or whether they are only abnormal appearances which stand outside the regular cycle. It is probably safer to describe the main types, recognizing that the divisions between them are arbitrary and to realize that the relation of one type to another is assumption rather than observed fact. Accordingly the following stages may be recognized.

1 *Small forms with one piece of chromatin* (Figs. 1 and 14).—These are usually rounded and measure about  $2.5\mu$  across. The chromatin is round or oval and measures 1 to  $1.6\mu$ —it stains pink or crimson with Giemsa. Sometimes the outer parts stain more clearly than the centre, sometimes the staining is uniform. The cytoplasm stains pale or dark blue with Giemsa—sometimes there are one or two small vacuoles in it. Often there is a small darkly staining

FIGS. 1 TO 3.—Parasites with one to four pieces of chromatin.

FIG. 4.—Parasite with five pieces of chromatin—cytoplasm rather granular.

FIG. 5.—Parasite with seven pieces of chromatin—cytoplasm is granular and contains vacuoles.

FIG. 6.—Schizont with many pieces of chromatin (many at other optical levels, not shown). The cytoplasm is beginning to be concentrated round some of the pieces of chromatin.

FIG. 7.—Schizont forming merozoites.

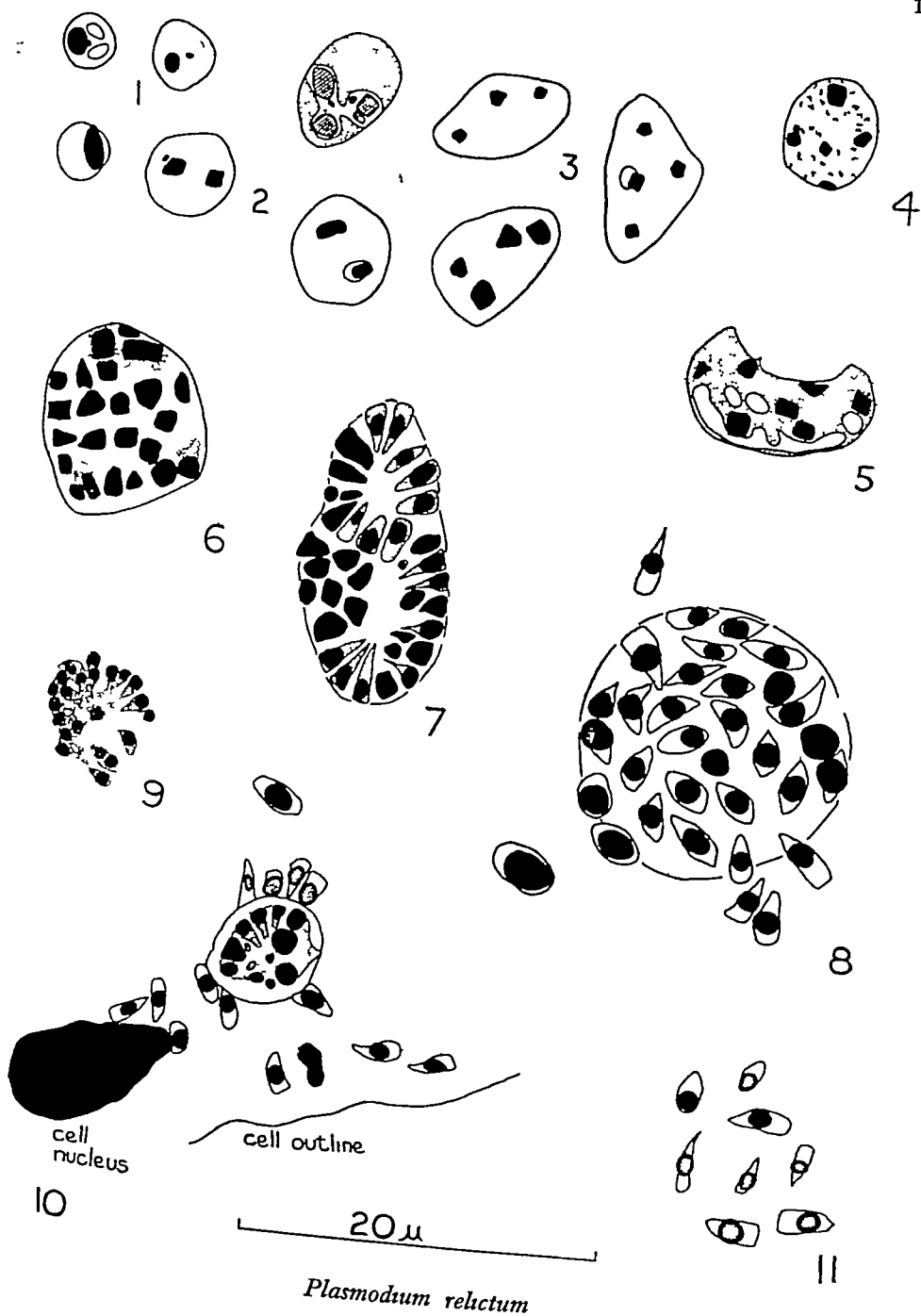
FIG. 8.—A mature schizont containing many merozoites arranged irregularly (others at different optical levels, not drawn)—some merozoites are emerging into the adjacent tissues.

FIG. 9.—A schizont breaking up into merozoites. ( $\times 1,900$  Drawn by V. D. M.).

FIG. 10.—A schizont at later stage of disintegration (see text).

F. G. 11.—A group of free merozoites. (Drawn by F. H.).

Magnification according to scale (except FIG. 9).





granule in the cytoplasm near the chromatin. (All recorded dimensions depend greatly on the degree of flattening of the parasite—flattened parasites naturally appear much larger under the microscope than those of equal volume but spherical shape. Since flattened parasites are easier to study and photograph than spherical ones, most of the illustrations and measurements are made on flattened ones.)

2. *Large forms with one piece of chromatin* (Fig. 15).—These are irregular in size and shape but often measure  $3.5\mu$  across. The chromatin is usually elongated ( $1.6 \times 0.8\mu$ ) but sometimes it is rounded. Often there is a clear space between the chromatin and the part of the cytoplasm which stains bright blue with Giemsa. A granule and vacuoles may be present. Presumably these forms represent the further growth of (1).

3. *Forms with several pieces of chromatin* (up to twenty or more), as illustrated (Figs. 2 to 5).—In the smaller forms the cytoplasm is abundant and stains bright blue. Granules and vacuoles may be present. Presumably these forms arise from the division of the chromatin shown in Fig. 2.

4. *Large schizonts with twenty or more pieces of chromatin* (Figs. 6, 12, 16).—These may be rounded or oval and may measure 9 to  $14\mu$  according to the volume and the degree of flattening. The number of pieces of chromatin ranges up to forty-five or more—the larger numbers being difficult to count accurately. These schizonts may be divided into two types. (a) With chromatin staining crimson with Giemsa and a fair amount of cytoplasm (staining bright blue) distributed approximately evenly between the pieces of chromatin—presumably these are a development of (3). (b) With chromatin staining more deeply and scanty cytoplasm which is concentrated closely round the piece of chromatin so that the intervening spaces appear empty (Fig. 6).

5. *Forms in which merozoites are foreshadowed*.—These forms are as big as those of (4). The pieces of chromatin stain dark crimson and each is surrounded with a little dense cytoplasm which may be rounded, or may be elongated as in a mature merozoite. These early merozoites may be arranged in three ways: (a) packed together apparently irregularly with many of the outer pieces lying tangentially. Some of these look as though the schizont had been converted into a cluster of merozoites arranged at random, which are gradually falling apart (Fig. 8). (b) with two clear areas surrounded by early merozoites, some spherical others elongated and lying radially. Some have twenty-four merozoites, some more (Figs. 7, 13). (c) elongated merozoites arranged radially round a small dark mass in the centre which might be chromatin or cytoplasm (difficult to distinguish) as in Fig. 9. This form has about twenty-one pieces of chromatin. Only one or two forms like this were seen.

The form shown in Fig. 10 is interesting. There are several merozoites lying free and several others arranged radially round a central mass which stained dark purple with Giemsa. Photographs revealed that this dark central mass had the structure shown in the drawing—there are eight or more pieces



FIG 12 —Large schizont    The longitudinal clear space is presumably an artefact  
Cultivated 18 days



FIG 13 —Three schizonts changing into merozoites    Cultivated 12 days

*Plasmodium relictum*  $\times 1,250$

granule in the cytoplasm near the chromatin. (All recorded dimensions depend greatly on the degree of flattening of the parasite—flattened parasites naturally appear much larger under the microscope than those of equal volume but spherical shape. Since flattened parasites are easier to study and photograph than spherical ones, most of the illustrations and measurements are made on flattened ones.)

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FIG. 13. Three schizonts (labeled) in interior of cyst. Cultivated 12 days.





FIG 14



FIG 15



FIG 16



FIG 17

*Plasmodium relictum*  $\times 1,250$

- FIG 14 —Two small forms with a single piece of chromatin Cultivated 19 days  
 FIG 15 —Many larger forms with single pieces of chromatin, other forms in adjacent cells Cultivated 12 days  
 FIG 16 —A large schizont and a smaller one Cultivated 12 days  
 FIG 17 —A group of merozoites in a large flattened cell Cultivated 12 days



of chromatin, some of which are partly differentiated into merozoites arranged radially. The appearances suggest merozoites being liberated in two successive crops from a central core, as suggested by HUFF and COULSTON (1945) for the meta-cryptozoites of *P. gallinaceum*. No other form was found in which this appearance was detected, but this failure may be due to the fact that most other forms, at this stage of schizogony, were overlaid by cells.

It is not clear whether (a), (b) and (c) are successive or alternative stages in the development of the parasite. In particular, it is difficult to reconcile form (a), in which the fully developed merozoites are arranged at random, with a cycle involving forms (b) and (c) in which the merozoites are arranged radially.

6 *Free merozoites* (Figs 11, 17).—These are elongated forms about  $0.8\mu$  wide by 2 to  $2.5\mu$  long. One end is pointed and the other is often rounded, or square. The chromatin is spherical, about  $0.8\mu$  across. Some authors have described macro-merozoites and micro-merozoites in the development of the exoerythrocytic forms of *P. relictum*. Only one type (as above) has been seen in these cultures.

## DISCUSSION

The main purpose of this investigation was to demonstrate that the tissue culture technique, which had been developed for the study of *P. gallinaceum*, was applicable also to *P. relictum*. It was pointed out in the previous paper (HAWKING, 1945) that any intracellular parasite, which lives inside a type of cell which can be grown *in vitro*, ought to prove susceptible to cultivation in this manner. The parasites which have now been grown in this way include *P. gallinaceum*, *P. relictum* and *P. lophurae* (TONKIN and HAWKING, in press), *Trypanosoma cruzi* (HAWKING, in press) and *Leishmania donovani* (HAWKING, in preparation for publication). In addition the growth of *P. cathemerum* in a single experiment was reported by HEGNER and WOLFSON (1939), and TCHERNOMORETZ (1945) has reported the cultivation of *Theileria annulata* by a technique which he had devised independently but which is similar to the one employed here.

Cultivation of parasites which grow in small animals (e.g., *Leishmania* in hamsters) or small birds (e.g., canaries) is much facilitated by the use of heterologous sera obtained from larger animals. In the present instance fowl serum was not completely satisfactory for the growth of the cells and perhaps the serum of some other species would have been better.

This technique is advantageous for studying the morphology of the exoerythrocytic forms, especially in the stages leading up to schizogony and the formation of merozoites. No clear description of these has been discovered in the previous papers on the exoerythrocytic forms of *P. relictum* (RAFFAELE, 1936, HEGNER and WOLFSON, 1938, MANWELL, 1940, REICHENOW and MUDROW, 1943), although some illustrations contain forms suggestive of



elongated merozoites. As described above, the merozoites of *P. relictum* assume their elongated form at an early stage of schizogony but the exact relationship of some of the forms seen, one to another is not clear. In the previous work on *P. gallinaceum*, a simpler method of fixing and mounting the preparations was used therefore, an attempt to compare the morphologies of the two plasmodia in tissue culture is not desirable in this place. REICHENOW and MUDROW (1943) described two types of merozoite (macro- and micro-merozoites) being formed during the pre-erythrocytic stages of *P. relictum* and HUFF and COULSTON (1945) noted two similar types in the pre-erythrocytic development of *P. gallinaceum*. In these cultures only one type of merozoite, corresponding presumably to macro-merozoites, was seen.

### SUMMARY

The exoerythrocytic forms of *Plasmodium relictum* have been grown in tissue culture, using the technique previously described for *P. gallinaceum*. Growth was terminated after 19 days. Fluid removed from the culture flasks after 5 and 9 days was infective for a canary. Growth took place in the presence of penicillin and of streptomycin. This technique is advantageous for studying the morphology of the exoerythrocytic forms, especially in the different phases of schizogony. The various stages are illustrated.

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## LATENCY AND LONG-TERM RELAPSES IN BENIGN TERTIAN MALARIA

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The phrase "a latent attack of malaria" is here used to denote a frank attack of fever associated with parasites in patients who were infected several months prior to the development of clinical symptoms. "A long-term relapse" denotes a clinical and parasitological attack at least 3 months after the primary attack, the latter having been treated with a course of mepacrine or quinine, and (as far as is known), the patient having remained fit and well, without parasites in the peripheral blood between the attacks.

It is my experience with therapeutic malaria that neither latency nor long-term relapses occur in malignant tertian malaria. This is based on a study of several geographical strains of both tropical and sub-tropical origin. M T malaria relapses, if they occur at all, do so at very short intervals, the first within a few weeks of the primary attack (and the others follow in quick succession). These, it is considered, are due to incomplete treatment of the previous attack in that not all the trophozoites were destroyed (radical cure). Latency, as seen in B T malaria, does not appear to occur in M T malaria. The incubation period in M T malaria varies between 6 and 18 days with an average of about 10 days. It is well known that infected persons who take small daily doses of a suppressive drug such as quinine may experience an attack of fever many weeks from the last date of infection, particularly when the drug is discontinued a week or two after infection. The ensuing attack in these cases is caused by circulating parasites which were not destroyed by suppressive treatment. Latency in B T malaria, as defined above, is known to occur with tropical, sub-tropical and temperate region strains, but there is some evidence to suggest that it occurs more frequently with temperate region strains than it does with tropical strains. Nothing is known about the biological factors concerned in true latency but we know under what circumstances it may occur. These are —

- I Under natural conditions
- II Due to drug prophylaxis

- III. In the presence of mixed infections—double infection (B T and VLT at the same time), the M T malaria developing is about 8 days and the B T malaria many months later
- IV. In patients who are immune to one strain and while retaining the immunity are subsequently infected with another strain.

### I LATENCY UNDER NATURAL CONDITIONS.

It is a well known phenomenon that in Eastern Europe there is frequently a sharp malaria curve in early spring caused by B T malaria. Many perhaps most, of these cases are primary attacks in persons who were infected in the previous summer and autumn. This phenomenon has been observed in Roumania, Italy, Spain, Holland and England, and according to KOTTREK (1902), and SWELLENGREBEL (1921), nearly all the indigenous malaria occurring in Northern Holland in the spring and summer is the result of infections contracted in the previous autumn. It may be of interest to record here some observations made at this laboratory concerning therapeutic malaria, because they bear testimony to observations made in the field.

The practice at this laboratory is to prepare batches of about 200 mosquitoes about once a month in order to supply B T malarial material to hospitals throughout the country for malaria therapy purposes. A patient undergoing malaria therapy whose blood contains the necessary number of ripe gametocytes is selected to infect a batch of mosquitoes. After the mosquitoes have fed once or twice they are incubated until sporozoites appear in the salivary glands, when they are ready for transmitting infection. Earlier in our work we continued to use the mosquitoes so long as we were able to find sporozoites in the salivary glands—this often exceeded a period of 2 months. It soon became apparent that the presence of sporozoites in the glands was not by itself evidence that the person bitten would develop malaria within the usual incubation period (10 to 14 days). It was seen that the majority of the patients who were bitten during the first 2 or 3 weeks following salivary gland infection of the mosquitoes, developed the disease within the normal incubation period. Many of the patients bitten later than this failed to develop fever for many months. Most of the failures were reinfected with a different species of parasite in order not to delay treatment. Others were reinfected by blood of the same species and a few not reinfected at all. Those patients who were infected with VLT or quartan malaria developed their first attack of B T malaria many months after the original infective bites despite the intervention of severe attacks of fever (caused by the different species).

We know that relapses do not occur in B T malaria induced by blood inoculations and as many of our mosquito-infected cases which failed to develop malaria within the normal incubation period developed fever several months later these attacks were due to the original mosquito-borne infection and were therefore latent cases.

When a batch of mosquitoes was infected on only two occasions, on alternate days, even though the infection was a particularly heavy one with the average number of oöcysts per insect being a hundred or more, cases bitten 4 to 5 weeks after gland infection failed to develop fever. It was observed that for the first few days following the invasion of the glands by sporozoites, not only were the gland cells packed with sporozoites but enormous numbers were found lying in the salivary duct. Following a number of blood meals, the gland ducts were free, or nearly free, of sporozoites, but the gland cells were still heavily infected. It is believed that this may have an important bearing on the degree of infectivity following biting. When the salivary ducts are packed with sporozoites, very large numbers would be discharged during the act of biting, but when the duct is free, though the gland cells are heavily infected, the numbers discharged would not be very great. After a heavily infected mosquito has bitten fifteen or twenty times, sporozoites can still be seen in the gland cells, even though, in many cases, the person bitten fails to develop malaria within the usual incubation period. In the early days of our work, after observing this phenomenon, it was concluded that the finding of sporozoites in the gland cells was not necessarily proof that the patient had been infected and we inclined to the opinion that under certain circumstances an infective mosquito may bite a person without injecting sporozoites. This may be true, especially in mosquitoes where the infection was a very light one, because the thoracic muscular pressure exerted during the act of biting may not have squeezed out any sporozoites embedded in the gland cells. But the point of interest here is that many of the believed-to-be-failures developed fever and parasites 6 months or even a year after infection and that during this long incubation period the infected individuals remained healthy. It is worthy of note that failures seldom, if ever, occurred when the patient was bitten by even one or two mosquitoes within a week or two after the glands first became infected. One other important factor in connection with this is worth considering. Might the failures be due to the age of the sporozoites and not to numbers? To test this a batch of infected mosquitoes which had been infected on only one occasion was fed on a rabbit over a period of 6 weeks. The actual number of times the mosquitoes bit the rabbit is not known but it is certain that they fed on at least twelve occasions. Dissections showed that the glands contained only very small numbers of sporozoites. A patient bitten by six of the batch failed to develop fever within 3 weeks. The glands of twelve mosquitoes of the same batch were dissected and injected intravenously into another patient and fever and parasites developed within the normal incubation period. In passing it should be understood that it is not intended to imply that a relatively small number of sporozoites *never* give rise to fever and parasites within the usual incubation periods, but it is considered that true latency occurs only when the sporozoites injected are too few to set up an immediate attack and that there is never true latency when large numbers of sporozoites are injected.

That true latency occurs in nature is so well known that it is not proposed to discuss it here. It has been studied and reported upon from many countries, particularly in Europe, and especially in Holland, where the fact that no species of human malaria parasites other than that of B T malaria occurs in nature, has afforded opportunities for a close study of latency to be made. The subject is dealt with fully by SWELLENGREBEL and DE BOCK (1938) in their book, *Malaria in the Netherlands*.

## II LATENCY DUE TO DRUG PROPHYLAXIS

### *Quinine*

Patients who take quinine daily beginning a day or two before exposure to infection and continuing for 6 to 8 days during the incubation period, usually have a slightly longer incubation period than is normally the case. Again, parasites are often difficult to find for several days even after fever has started. On the other hand, if quinine is given a day or two before infection and continued daily for 2 or 3 weeks, there is usually a single peak of fever only. If a prolonged search is made, an odd parasite may be found. In many of these cases the patient remains symptom-free for several months and then he may experience a severe attack of malaria. In such cases it is believed that the quinine has acted in the usual therapeutic manner that the asexual parasites have been destroyed and the subsequent long-term relapse is characteristic of the species of parasite. It is significant that the interval between the primary attack and the first relapse is comparable to a prolonged incubation period where there has been no primary attack.

### *Pamaquin (Plasmoquine)*

This drug which has very little therapeutic action on the asexual parasites of B.T. malaria, is capable of warding off a primary attack for several months, even though the drug is stopped several days before the termination of the normal incubation period. In nearly every case, however, an attack develops several months later and fever and parasites resemble in all respects a primary attack when the incubation period is normal. Following the treatment of a primary attack with a protracted incubation period, relapses may occur in some cases only once, while in others there may be several at short intervals of a month or so. It is of interest to mention here that pamaquin is equally effective as a prophylactic against M.T. malaria, but with this species of parasite, if the drug successfully prevents the primary attack, there are no subsequent attacks either at short or long periods. Therefore pamaquin in certain doses when given on the day of infection and for a few days afterwards, sterilizes an infection of M.T. malaria before fever or erythrocytic parasites appear. It is equally effective against the primary attacks of B.T. malaria but in this case the disease manifests itself many months later. It is believed that this difference is not

related to the action of the drug on the erythrocytic parasites, but is due to differences in the biology of the species

### *Mepacrine (Atebrin)*

It is of great interest and importance to find that this drug has the same effect as pamaquin. When it is used prophylactically against certain strains of M T malaria, unless fever and parasites occur within about the usual incubation period, no attack develops. With B T malaria there is nearly always the same latent infection as when pamaquin is used. One important point in these B T malarial cases is that although the drugs are given for only a few days at the time of infection, the infected person remains well for several months. This is entirely different from what happens when quinine is given for the same period.

### III LATENCY IN THE PRESENCE OF MIXED INFECTIONS

Only a few cases of this series are available for study, but they are worthy of consideration.

Patients may be infected with B T and M T malaria at the same time, either by mosquitoes containing sporozoites of both species of parasites or by mosquitoes, some of which are infected with B T malaria and others with M T. Fever occurs some 10 days later but only M T parasites are found in the blood. If, after about a week of continued fever, drugs are given which successfully abort the attack but fail to cure the disease, two or more relapses due to the same species may occur over a period of 3 or 4 months. No B T malarial parasites are seen and clinically and parasitologically the disease is due to one species only—that of M T malaria. Several months later the patient again develops fever, but this time it is due to B T malaria, and only parasites of this species are to be found in the blood. On the other hand, if a patient is infected with B T malaria 3 or 4 days before being infected with M T malaria, the parasites of both species appear in the blood together, and continue to do so until treatment is given. (It was observed that in mixed infections gametocytes of both species are usually very numerous.)

### IV LATENCY IN PATIENTS WHO ARE IMMUNE TO ONE STRAIN, AND WHILE RETAINING THIS IMMUNITY ARE SUBSEQUENTLY INFECTED WITH ANOTHER STRAIN

Some patients who had received a full course of malaria-therapy with our Madagascar strain failed to develop fever or parasites when they were several times reinfected with the same strain of the same species, either by blood or mosquitoes, they were immune. But if they were infected with a different strain of the same species, some developed fever and parasites but with a protracted incubation period, usually of several months duration. If, however, they were infected with a different species of parasite, fever and parasites

developed within the usual incubation period and the attacks were just as severe as is normal for the species.

In one series of experiments, a patient, a primary case, was infected by blood inoculation with a strain of B T malaria from the French Cameroons, and from him a batch of mosquitoes was infected. The mosquito infection was a light one and in none of those dissected were there more than eight oöcysts per stomach. Six patients who were immune to the Madagascar strain were bitten by mosquitoes of this batch, and although the infection seemed to have failed all the patients developed fever and parasites several months later. The following summary gives the history of the six cases in which two strains of B T malarial parasites were used—Madagascar and French Cameroon strains.

#### Case 1

- 1927 Primary infection with Madagascar strain. Ten peaks of fever treated with quinine.  
 1931 Second infection with same strain. Parasites without fever.  
 Third exposure to infection 3 months later—failed.  
 1932 Fourth exposure to infection 9 months later—failed.  
 Five months later infected by mosquito bites, French Cameroon strain. Fever and parasites developed 296 days later. Treated with quinine. There were two relapses, first 1 month after end of treatment, second 2 months after end of first relapse.

#### Case 2

- 1928 Primary attack with Madagascar strain. Ten attacks, then treated with quinine. Relapsed 5 months later.  
 1931 Second infection with same strain. Fever and parasites followed by spontaneous recovery.  
 Third exposure to infection 1 month later. Bitten by over 300 infected mosquitoes—failed.  
 Fourth exposure to infection 1 month later. Bitten by over 100 mosquitoes—failed.  
 1932 Fifth exposure to infection 9 months later—failed.  
 Two months later bitten by mosquitoes infected with French Cameroon strain. Fever and parasites developed 263 days later. Treated by quinine. There were no relapses.

#### Case 3.

- 1928 Primary attack with Madagascar strain. Ten attacks then treated with quinine. Relapsed 8 months later.  
 1928 Second exposure to infection—failed after being bitten by 140 infected mosquitoes.  
 Third exposure to infection, bitten by 150 mosquitoes—failed.  
 August, fourth exposure to infection, bitten by fifteen mosquitoes—failed.  
 1929 Fifth exposure to infection, bitten by thirty mosquitoes—failed.  
 Sixth exposure to infection, bitten by forty mosquitoes—failed.  
 Seventh exposure to infection, bitten by twenty five mosquitoes—failed.  
 December exposed to infection with VLT malaria (successful).  
 1930 Eighth exposure to infection, again bitten by mosquitoes infected with Madagascar strain—failed.  
 1931 Ninth exposure to infection, bitten by one hundred mosquitoes—failed.

1932 Tenth exposure to infection, bitten by fifty mosquitoes—failed  
In December, 1932, bitten by ten mosquitoes infected with French Cameroon strain Fever started 253 days later and continued for 10 days when treatment was given There were no relapses

## Case 4

1929 First infection—with Madagascar strain  
1932 Second exposure to infection—failed  
In December, 1932, bitten by eleven mosquitoes infected with French Cameroon strain Fever started 287 days later and continued for 10 days There were no relapses

## Case 5

1926 First infection—with Madagascar strain.  
1927 Second exposure to infection—failed  
Third exposure to infection—failed  
1931 Fourth exposure to infection—attack started but spontaneous recovery occurred after 4 days of parasites  
1932 Fifth exposure to infection—failed  
Sixth exposure to infection—failed  
Eight months later, infected with French Cameroon strain Incubation period 304 days Fever continued for 10 days and patient was treated with quinine There were no relapses

## Case 6

1926 First infection—with Madagascar strain  
1932 Second exposure to infection 4 months later—successful  
Third exposure to infection—failed  
Bitten by twelve mosquitoes infected with French Cameroon strain  
Incubation period 311 days Fever and parasites for 12 days Treated with quinine There were no relapses

In this small series of cases of B T malaria all the patients were immune to the Madagascar strain As the number of oöcysts in the stomachs of the mosquitoes infected with the French Cameroon strain was small, the sporozoites injected would be relatively few

Two primary cases were infected with the French Cameroon strain by direct blood inoculation and both developed fever and parasites within the usual incubation period

Four cases immune to the Madagascar strain were infected by direct blood inoculation with the French Cameroon strain and all developed fever and parasites within the normal incubation period The two primary cases continued to have fever until treatment was given, whereas the immune cases all had spontaneous recoveries after a few days

## TIME FACTORS

## 1 True Latency

In nine patients infected with B T malaria (observed by the writer) who failed to develop fever and parasites for several months, but who developed fever and parasites subsequently without reinfection, the average number of days



between infection and the attack was 282. On examining the findings of other workers in Europe, similar results are seen. SWELLENGREHL and DE BOCK (1938) record (page 150) an experiment where eight volunteers were infected by one or two mosquitoes. All eight developed true latent malaria, one with an incubation period of 231 days a second with 270 days and the remainder with incubation periods varying between 239 and 276 days.

### 2. Latency brought about by Drug Prophylaxis

In latency brought about by drug prophylaxis, e.g. mepacrine or pamaquin, the interval is about the same. In nineteen carefully selected cases of B.T. injection the incubation period averaged 263 days.

### 3. Long-term Relapses

One of the greatest problems of B.T. malaria is the prevention of relapses. It is generally believed that they are due to insufficient treatment of the primary attack or previous relapse. Such is undoubtedly the case when, as the result of insufficient treatment, a primary attack is followed by a short term relapse (recrudescence) some few weeks later. This can be demonstrated by giving a single dose of 10 grains of quinine to a patient in the throes of a primary attack with certain strains of B.T. malaria. Fever subsides after about 3 days and parasites can seldom be found in thick smears by the 5th day. But this fever free interval is of short duration and by the 14th day fever begins again and parasites are numerous in the peripheral blood. The single dose of the drug has aborted the attack and reduced the number of parasites below the pyrogenic threshold. Some parasites survive and in the absence of further treatment they soon increase in number sufficiently to cause clinical symptoms. There is, however, some reason for believing that long-term relapses are not due to the survival of a few asexual parasites. It is of significance that the time factor in long-term relapses is closely related to that of true latency and of latency brought about by drug prophylaxis (mepacrine and pamaquin but not quinine). In fifty-two carefully selected long term relapses the average period of time from the last attack was 263 days.

Two examples may be quoted

(1) In August, 1943 two indigenous cases of B.T. malaria occurred in male patients in a mental hospital in Surrey. The patients shared the same ward, neither had ever been abroad nor subjected to therapeutic malaria. One patient developed B.T. malaria on 22nd August and the other on the following day. Parasites were present in the blood of both cases in moderate numbers.

*Type of fever*—In one patient the fever was quotidian and in the other it was tertian.

*Treatment of the attacks*.—Both patients were treated with mepacrine 0.3 grammes daily for 7 days and fever and parasites quickly disappeared.

In April of the following year both patients relapsed, clinically and parasitologically. In one, the interval between the primary attack and the first relapse was 238 days and in the other 229 days. Although there were more than twenty patients sleeping in the ward, no further cases occurred and it is conjectured that a single mosquito infected both patients within a few days of each other.

(2) In September, 1935, a case of indigenous B T malaria occurred in a young woman aged 36, living in Essex. Three weeks later a second case occurred in another house, this time in a boy aged 12, who shared a bed with his grandmother at week-ends. Eleven months later the grandmother developed malaria. The two houses were separated from each other by about 100 yards. It is believed that the three cases were infected by the same mosquito within a few days of each other, that the first case was infected about the middle of August, and that the mosquito left the house, probably to lay eggs, and later returned to the house where the other two cases occurred. The boy was probably the next to be infected and he, too, developed the disease within the normal incubation period. The third case was probably the last to be infected. It is considered that the infected mosquito discharged a sufficiently large dose of sporozoites in the first two cases to produce symptoms within the usual incubation period, but that the third case received a minimum number of sporozoites, and that this is the reason for the protracted incubation period, *i.e.*, 9 to 11 months.

As a result of some recent experiments where the number of sporozoites injected is known, there is some evidence which suggests that about 2,000 sporozoites are necessary to ensure a normal incubation period. In a recent experiment the glands of five mosquitoes were dissected in Locke's fluid and an emulsion prepared. Two patients were infected, one receiving 371,300 sporozoites and the other 1,805. The patient receiving the large dose developed fever in 10 days while the patient receiving the smaller dose failed to develop either fever or parasites and was subsequently infected by direct blood inoculation with the same strain and started fever within a week. If the number of B T malarial sporozoites necessary to produce clinical symptoms is in the region of 2,000 it is not difficult to understand why so many cases are latent but it is difficult to understand why so many of these latent cases, even the majority, have incubation periods of about 38 weeks, at least with certain strains of tropical and sub-tropical origin.

#### EFFECT OF DRUGS ON THE MORPHOLOGY OF PARASITES

While some workers claim that they are able to distinguish quinine-affected parasites from the normal, others have failed to do so. Some years ago this was tested. I selected two patients who showed a heavy infection of B T malarial parasites and each was given 90 grains of quinine orally, one dose of 45 grains at 10 a.m. and a second dose 4 hours later. A series of thin films was taken at 2-hourly intervals over a period of 4 hours and stained with Leishman. Films of patients who had not been given quinine were prepared at the same time

intervals and were stained together. The two sets of films were then examined by workers who were authorities on parasitology but all failed to distinguish which of the films contained quininized parasites. No such problem presents itself with atebriinized parasites. Following the administration of a single dose of 0.6 grammes of atebriin (mepacrine), changes in the parasite can be seen within 20 minutes. Normally a full-grown asexual B.T. malarial parasite contains about 50 granules of pigment. Following a single large dose of atebriin, the first change which can be seen in the parasite is clumping of the pigment. In the first half-hour instead of there being about 50 granules of pigment, there are about twenty clumps. An hour or two later the number is further reduced and usually consists of two or three lumps. After about 12 hours many of the parasites are devoid of pigment and some early disintegration of both chromatin and cytoplasm can be seen. (I have failed to observe where the pigment goes. pigmented mononuclear cells in the peripheral circulation are not increased.) At about 24 hours disintegration is well marked and often within 48 hours no parasites can be found in thin films. As far as my observations go I have never seen a parasite which, after a dose of 0.6 grammes of atebriin, conforms to the normal. It is, therefore, possible that atebriin given over a period of several days may succeed in killing all the asexual parasites. Yet it is the case that many patients treated with this drug continue to relapse at relatively short intervals over a period of several months.

There is considerable evidence that plasmoquine (pamaquin), combined with quinine, reduces the relapse rate in B.T. malaria and many workers believe that this form of treatment is superior to either quinine or atebriin. When it is remembered that plasmoquine has very little action on B.T. malarial trophozoites at least when compared with quinine or atebriin, it is difficult to understand why plasmoquine in very small doses may reduce the relapse rate. Plasmoquine, when given a day or two before infection and continued for a few days afterwards, prevents the onset of a primary attack of B.T. malaria for several months, sometimes for a year. In M.T. malaria plasmoquine is even more successful and seems to be true causal prophylactic. If this is so then the indication is that against M.T. malaria, plasmoquine is either a sporozoicidal drug or that it acts on the hypothetical stage of the parasite between the sporozoite and the erythrocytic parasite. What then, is the difference between M.T. and B.T. malaria in this connection? In B.T. malaria, as has already been noted an attack is often delayed for nearly a year even when the drug is given for only a few days following exposure to infection. A possible explanation is that plasmoquine kills all the sporozoites or x bodies in a M.T. infection, but not all in a B.T. infection. Even if this is so it would still not explain the very long interval between infection and symptoms in B.T. malaria. If however the sporozoites, after escaping from the peripheral circulation, succeeded in establishing themselves in reticulo-endothelial cells and were able to outlive the life of the host cell they might, on being released, give rise to that

stage of parasitism which brings about infection of the erythrocytes. Whatever the explanation of latency, it would appear that it is caused by a *resting* parasite and not one which is active, such as a trophozoite. In advancing the theory that it may be the sporozoite itself which is lying dormant all these months, it is of interest to note that in the insect host B T malarial sporozoites are able to lie dormant for several months without, apparently taking nourishment or changing their morphological character even within a wide range of temperature.

The discovery of exoerythrocytic parasites in certain avian malarias, especially *P. gallinaceum* in the domestic fowl and *P. lophurae* in turkeys, has stimulated malaria research workers to attempt to demonstrate a similar cycle in mammalian malaria. From the literature it appears that these exoerythrocytic parasites are pathogenic and that in some cases they cause death to the host even before the later stages (erythrocytic parasites) have had time to develop. It would therefore seem unlikely that these forms of the parasite, even if they occur in B T malaria, are responsible for relapses. Furthermore, in B T malaria, relapses do not occur in blood-inoculated cases whereas exoerythrocytic parasites do occur in blood-inoculated cases of *P. gallinaceum* or *P. lophurae*. Some workers have shown that a single dose of 1 gramme of quinine given orally will sterilize a primary infection of B T malaria induced by blood inoculation whereas the same quantity of quinine given daily for 30 days will not prevent a long-term relapse in mosquito-infected cases with the same strain.

#### THE EVALUATION OF DRUGS IN ANTI-RELAPSE TREATMENT

It is a fundamental characteristic of many strains of B T malaria that between the primary attack and the first relapse there is usually an interval of several months, but, as far as is known, the interval never exceeds 1 year. Following the first relapse, there is frequently a series of relapses which occur at about 1-monthly intervals and these may continue over a period of from 1 to 2 years. Are these short relapses caused by erythrocytic parasites (trophozoites), which escaped destruction during treatment of the previous relapse? If sporozoites of B T malaria in human tissue cells are able to survive for many months as they are able to do in the tissue cells of a mosquito, it seems possible that they may be the direct cause of relapses. This could conceivably be the reason why one or many relapses recur at short intervals following an interval of many months up to 1 year between the primary attack and the first relapse. This phenomenon in B T malaria must have a very important bearing on the value of anti-relapse drugs. A patient who has had his first relapse 6 months to 1 year following his primary attack, followed by a number of short-term relapses, for about a year after the first relapse, will be semi-immune and such cases are not, at this stage of their disease, suitable for testing anti-relapse drugs. It is also the case that because so many patients infected with certain strains of B T malaria do not experience a relapse for from 6 months to 1 year following

the primary attack, the value of a drug as an anti relapse treatment cannot be properly assessed for at least 1 year after the primary attack.

*If relapses in B T malaria are caused by sporozoites or by an intermediate stage—between the sporozoite and the erythrocytic stages of the parasite—a drug which will prevent relapses would also conceivably be equally successful as a causal prophylactic and vice versa.*

### SUMMARY

The first relapse in B T malarial infections usually occurs several months after the treatment of the primary attack, and may be as long delayed as 1 year.

A case is presented for considering that relapses are due to sporozoites which have been held up in tissue cells, a not improbable hypothesis when it is remembered that sporozoites are able to survive for many months in the tissue cells of the insect carrier.

True latency is seen in those cases in which the primary attack occurs many months after infection, frequently after a delay of about 38 weeks. (The delay varies between 2 months and 1 year.) First relapses frequently occur at about the same period of time.

Latency as defined in this paper occurs (1) in nature (2) following certain drug prophylaxis (3) when mixed infections of B.T. and M.T. malaria occur (4) when patients who are immune to one strain of B.T. malaria are infected by a different strain.

The significance of the drug pamaquin (plasmoquine) is that it prevents the onset of an attack for many months, whereas, compared with quinine and mepacrine in an acute primary attack its action on erythrocytic parasites is slight.

It is suggested that relapses in B.T. malaria are due to a *resting* phase of the parasite and that a drug which successfully prevents relapses would also act as a successful causal prophylactic and *vice-versa*. Sporozoites are known to survive for many months in the tissue cells of the insect host and it is suggested that they also may be able to do so in the human host.

The word "prophylaxis" has been used in this paper deliberately in preference to the word "suppressive". When quinine is used as a prophylactic against B.T. malarial infections, if the drug is discontinued a few days after exposure to infection, the onset of fever is delayed for at most a few days. When mepacrine or pamaquin is used in one strain at least (Madagascar strain) fever seldom occurs within 2 months of infection and usually the period is between 34 and 38 weeks. It is, therefore, considered that while the word suppressive is applicable to quinine it is less appropriate to mepacrine and pamaquin.

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## CORRESPONDENCE.

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### NEED FOR SOCIAL SERVICES IN THE TROPICS

*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

Dr L EVERARD NAPIER's paper\* on the teaching of tropical medicine contains many statements of great importance. He summarizes his two main points as follows —

1 The necessity for making the undergraduate student conscious of the existence of diseases other than those that commonly occur in his own country

2 The urgent need for a hospital in London that will act as a clinical centre for teaching and research in tropical medicine

With the second of these I heartily concur. With the first I also agree, but would like to have it differently expressed. If environmental medicine were properly emphasized, if the aetiology of disease were studied, there would be no need for any cleavage between "medicine" and "tropical medicine". The doctor would then automatically pay attention to conditions and diseases in any locality in which he was stationed. "Tropical medicine" has been an unfortunate term, because it has led doctors to believe that certain diseases depended on the degree of latitude, whereas they were rather determined by ignorance, poverty, food, housing and toilet habits of a people. The effect of heat and humidity on the imported Europeans has been studied extensively, while the effect of separation from his children or his parents—the effect of a new intellectual and social atmosphere—has been studied very little. I would ask Dr NAPIER to restate his first item something like this: "The

\* NAPIER, L. EVERARD (1946) Teaching of Tropical Medicine *Trans R Soc trop Med Hyg*, 39, 273

necessity for making the undergraduate student conscious of the importance of environment to disease." This statement is capable of elaboration. I shall try to elaborate it by referring to Dr NAPIER's suggested syllabus and staff.

The syllabus contains no mention of social services. These are becoming more and more recognized as indispensable handmaids of medicine in temperate climates, why not in the tropics where there is a much greater incidence of *preventible disease*? It is possible to control the major epidemic diseases by mass vaccinations, water purification and port sanitation, but the amount of mortality and morbidity produced by these diseases is generally small compared with that caused by ignorance, poverty and unhygienic habits, and it is only by improving and extending social services and health education that this can be effected.

In the past the medical services in undeveloped countries have tended to provide (1) hospitals—sometimes amazingly good hospitals—and treatment for the individual sick and (2) public health measures—sometimes amazingly good public health measures—to control major epidemic diseases in an impersonal way. There now remains a great mass of preventible disease which can only be approached and treated (or prevented) in a personal way. These are the places where the health nurses and social service workers, if *properly trained, supervised and organized by the doctor*, will do more to improve health and to save life than any amount of study of "herpetology" and "obstetrics in the tropics." And if such social service is properly organized it will save a great deal of money that would otherwise be spent on doctors and hospitals, drugs and patent medicines.

It is true that social services and welfare work in the tropics are lamentably stereotyped and under-developed, but there is no need for this to be perpetuated. There is nothing that is more important for the student to learn than the scope and application of these services, their organization and the training and supervision of the staff.

Colonel E. H. VERR HODGE remarked that some attention must be paid to the feeding and care of infants in the tropics. This is the only reference I can find to the subject of child health. Dr NAPIER does not include paediatrics in his syllabus nor a paediatrician on his staff. I should like to see these omissions remedied or know the reason why.

Lastly may I point out that both the environmental aspect of medicine and the subject of child health are included in welfare work. The study of the various possible methods and applications of welfare work, in its widest sense, would do much to improve the minds of those who study medicine and the well-being of their patients.

I am, etc.,

(Sgd.) CICELY D. WILLIAMS, D.M.

Singapore.

## THE EPIDEMIOLOGY OF CHOLERA

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

In an Annotation (1943) in the *British Medical Journal* on "The nature of the influenza virus," the writer remarks that one of the great mysteries in the epidemiology of influenza is the mechanism whereby the virus survives through long inter-epidemic periods

He refers to the work of SHOPE on swine influenza, particularly to his demonstration that swine influenza virus while in the lung-worm, exists in a masked form and is totally non-infective until awakened to activity by some provoking stimulus

He states that SHOPE thinks that the onset of a swine epizootic is determined, not by the acquisition of the causative virus, but by meteorological or physical conditions which favour virus activation, and he expresses the opinion that it would be unwise to ignore the possibility of a similar mechanism existing in human beings \*

Finally, he points out that human epidemics, like swine epizootics, usually occur during a particular season of the year and that epidemic foci often arise simultaneously and apparently independently, suggesting some activation of previously acquired infection rather than direct case-to-case transmission

In their joint investigation (1925-27) into the bacteriology and epidemiology of cholera in the Asansol Mining Settlement, Bengal, on behalf of the Indian Research Fund Association and the Calcutta School of Tropical Medicine, TOMB and MAITRA arrived at a conclusion regarding the epidemiology of cholera in close accord with that subsequently arrived at by SHOPE regarding swine influenza

In an article entitled "A new conception of the epidemiology and endemiology of cholera" (1927), they reported that under the meteorological conditions obtaining in the mining settlement during the hot dry weather (May) Koch's agglutinating (epidemic) vibrio, when inseminated into a ground tank (pond) in the form of a cholera stool, lost its specific agglutinability after 12 to 14 hours

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\* The mechanism by which infectivity is increased or diminished in a virus is thus described by FENTON (1945) —

"Our best clue to the nature of gene mutations is furnished by viruses, those sub-microscopic germs that cause colds, influenza, yellow fever, and a number of diseases in plants. They, too, are giant molecules containing hundreds of thousands of atoms that are linked in complex groups and series. For generations these molecules may be stable, then they abruptly change. Thus the virus which normally causes tobacco mosaic may add a few thousand atoms and become a variety that produces the much more virulent ailment known as acuba. Other modifications may cause a disease that kills the affected plant or make the virus so weak that its effects can barely be detected. Apparently a single virus strain may appear in any or all of these forms, shifting from one to another as atoms are gained or lost."

An analogous mechanism in bacteria also may not unreasonably be inferred



They also reported that they had found that at least one third of the permanent inhabitants of the mining settlement (an endemic area) were constant carriers of non-agglutinating (non-epidemic) vibrios.

In conclusion they wrote as follows —

"We have therefore been driven to the unavoidable conclusion that the non-agglutinating (non-epidemic) vibrio takes on the agglutinating (epidemic) characteristic under certain biochemical-physical conditions [often seasonal] in the human intestine, the nature of which are at present unknown, and in this mutation or epidemic form is the cause of epidemic cholera, since it is not unreasonable to assume that characteristic is unstable may as easily be acquired as lost. Non-agglutinating (non-epidemic) intestinal vibrios, therefore, in our opinion constitute the reservoir of cholera both epidemic and endemic.

In a previous article (1926) they also wrote —

During our investigations in the Asarnool Mining Settlement we have met with several outbreaks of epidemic cholera in distant and isolated villages, the inhabitants of which had not been in contact either recently or remotely with any case of epidemic cholera. Spontaneous outbreaks of epidemic cholera have also been noted by other observers in other localities. Hitherto the explanation of such outbreaks has been that they owed their origin to some carrier of Koch (epidemic) vibrio who existed unknown in the community. Our suggested explanation of such outbreaks now is that owing to favouring circumstances [often seasonal] the non-agglutinating (non-epidemic) vibrio changes into the agglutinating or epidemic form in one of the numerous chronic carriers of non-agglutinating vibrios [in endemic areas such as the Mining Settlement], the epidemic spreading in the usual manner by contamination of water and by contact.

I am, etc.,

J. WALKER TONIB

Sydney New South Wales.

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It was necessary in the first place to have a picture of the whole course of the disease in order to correlate the bone lesion with its various stages. Dr HACKETT returned to this country just before the war with a mass of material on all aspects of yaws but was not able to deal with it because he was occupied in various parts of the world with duties in the Royal Air Force, which he had joined. The paper we are to hear this evening is the first public communication Dr HACKETT will have made on the work he did in Uganda over a period of two years. He will describe to us the general course of the disease illustrating his remarks by a number of lantern slides. Immediately after the paper he will show his film entitled 'Yaws in Uganda'.

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## PAPER

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### THE CLINICAL COURSE OF YAWS IN LANGO, UGANDA

BY

C. J. HACKETT

*Director, Wellcome Museum of Medical Science*

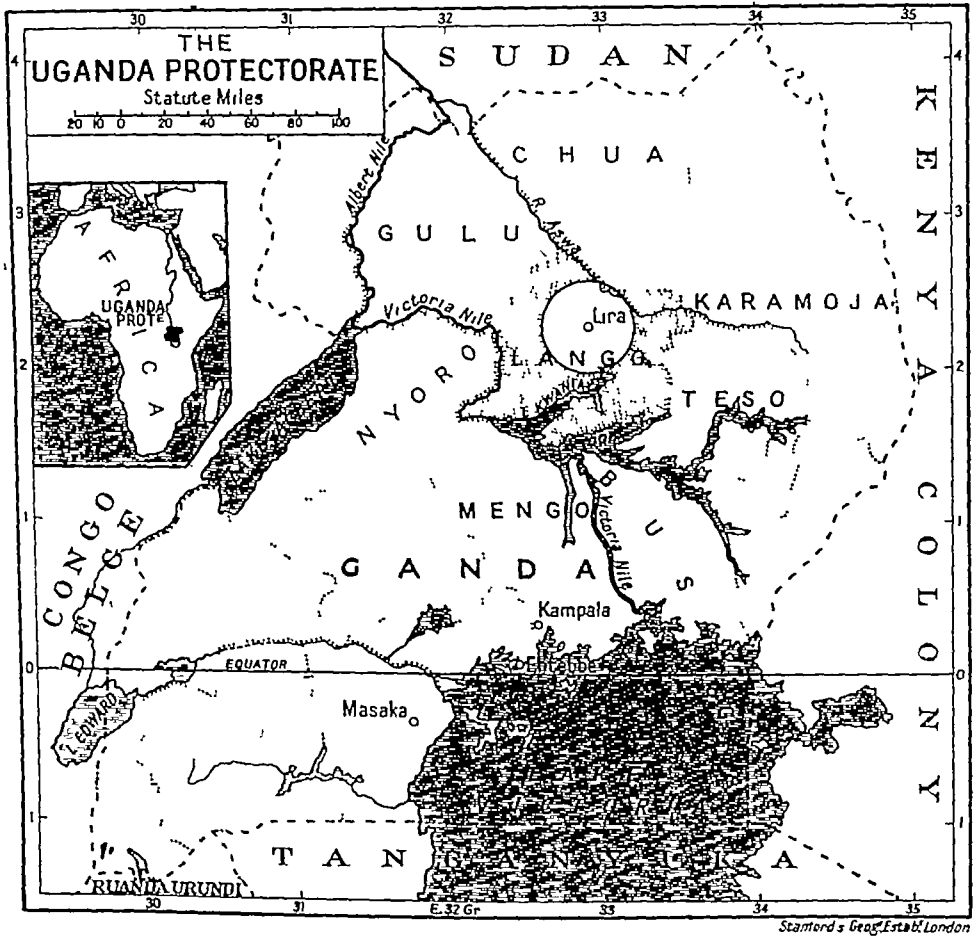
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Any study of yaws raises several questions. One is concerned with the relationship of yaws and syphilis. The most recent unitarian view of treponematoses is that of HUDSON (1948). He maintains that only one organism is concerned, *Treponema pallidum*, with two main, but not static, varieties which cause venereal and non-venereal treponematoses. One may evolve into the other providing environmental conditions are favourable. The time required for such a change is from a few years to several centuries. Such an advanced view will not be generally accepted without more evidence in its support. Until then yaws and syphilis may well be regarded as separate diseases as STANNUS (1936) and STOKES *et al* (1943) indicate.

A study of certain bone lesions in Australian aborigines (HACKETT 1936) showed the need for further information on the bone lesions of yaws. It was during a study of the bone lesions in 400 cases of yaws for periods up to 18 months at Lira (Lango district, Uganda) that the observations upon which

The material for this paper was obtained during 1937-40 while holding Senior Fellowship in Tropical Medicine of the Medical Research Council. I am indebted for much help to Dr W. H. KAUFMAN, then Director of Medical Services, Uganda, and his medical officers.

this paper is based were made. This, at once, raises another question, whether the condition studied was yaws or syphilis. At Lira (see map) yaws was one of the most prevalent causes for out-patient attendances, and syphilis was rare (see Diagram 1). At Masaka, in a different tribal (Ganda) area, the reverse was present (HACKETT, 1946a). Among the characteristics of yaws at Lira were a high childhood incidence of papillomatous lesions and an absence of

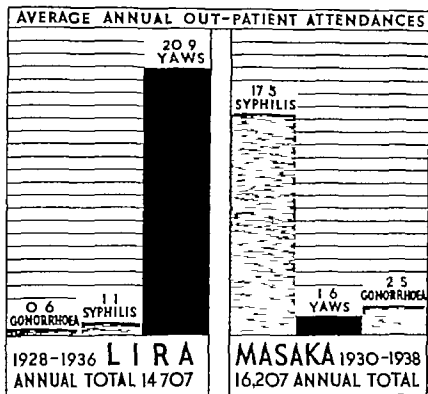


primary genital lesions in children and adults. Among those of syphilis at Masaka were an absence of such generalized eruptions but a high incidence of primary genital lesions in young adults. Some tertiary skin lesions of yaws and syphilis probably cannot at present be differentiated clinically. However, assuming that yaws is a separate disease, it may be accepted that the disease studied at Lira was yaws.

## THE STAGES OF YAWS.

Before dealing with the clinical course of yaws it is as well to define the various stages through which the disease may pass. This is necessary on account of the confusion in some recently published clinical descriptions. The Lango name for yaws is *nyach*.

DIAGRAM 1



## THE INITIAL LESION

At the site of infection which is some insignificant breach in the skin, or less often an existing ulcer the initial lesion develops (Fig 1). Usually when patients seek treatment this lesion either is associated with the generalized secondary eruption or has already healed. Some African communities, e.g. the Lango identify the primary yaws as the "first yaws" and when present alone they may diagnose it by its appearance and frequent association with bone pain. The initial lesion resembles typical secondary yaws, but is often larger and sometimes does not heal spontaneously as readily as do the secondary

eruptions Those which have been implanted on pre-existing ulcers are usually more luxuriant Specific therapy will lead to the healing of the yaws lesion, but the pre-existing ulceration often remains This may account for descriptions of ulcerating types of initial lesions

#### THE SECONDARY STAGE

Of all yaws lesions, that most generally recognized is the scattered eruption over the surface of the body of usually raised, apparently granulomatous papules (Fig 2) These papules vary in size from a few mm to 50 mm or more in diameter The smaller ones are round, but the larger ones are oval or elongated, their shape probably depending on the movements of the skin The surface, although granular in appearance, is not true granulation tissue, but is made up of greatly proliferated epithelium This is thrown into minute (less than 1 mm) elevations, where the prolongations from the corium approach the surface These minute projections are a pale yellow colour tinged by small dilated superficial blood vessels Often the minute contours of proliferated epithelium separating these elevations are much paler, or even white, so that an arabesque tracery appearance results The drying of exuded clear serum, in which spirochaetes of the syphilis/yaws type are readily demonstrated, produces a surface glaze This is the appearance of an actively developing lesion With decrease in activity a crust develops which, while yellow at first, soon becomes discoloured by debris In younger children with anaemia or malnutrition the lesions may not be raised, but are erosions with bright pink borders and whitish centres (Fig 3) Other clinical forms such as circinate and rupial lesions may occur These lesions are the "pianomes" of MONTEL (1944)

The number of lesions present varies from a single one to hundreds The trunk may be relatively free and the scalp is not frequently affected Since the surface of these lesions is largely proliferated epithelium, on healing only slight scarring may result There is often, however, an alteration of texture with loss of elasticity At first the scar may be more pigmented than the surrounding skin (Fig 4), but within a few months the pigmentation usually fades The scars are never permanently atrophic and pigmented, as in the tertiary lesions

This is the most frequent yaws lesion and is characteristic of the secondary stage It is present with, precedes or follows all the atypical secondary yaws skin lesions

#### THE TERTIARY STAGE

Another group of skin lesions occurring in yaws is characterized by destruction and ulceration They appear in two forms (a) an extensive superficial relatively clean ulceration (Fig 5) with a spreading edge and later a healing centre, or (b) a lesion which commences as a cutaneous or subcutaneous induration (Fig 6) and breaks down to form a localized indolent ulcer whose

base is coarsely irregular (Fig. 7). Specific spirochaetes cannot usually be demonstrated. In healing the epithelium grows in from the edges or from isolated central areas and atrophic scars (Fig. 8) result. These may be unpigmented in the earlier stages, but later are often deeply pigmented and may give rise to contractures. Secondary lesions of the skin or bones are never observed at the same time or later. Although these ulcers are given particular names in some African communities and separated from other types of ulcer it is probable that they are not recognized as yaws in such communities. In Lango they are known as *angwal* or *stoko*.

Since these destructive lesions always follow the secondary eruption, usually after a relatively or completely symptom-free interval of several years they must be regarded as belonging to the tertiary stage of the disease.

Having thus defined the characteristic skin lesions of the secondary and tertiary stages, other lesions may be grouped according to their association with one or other of them.

#### LATENT YAWS.

At Lusa (Uganda) many patients, according to their statements and by observation, some time after the resolution of their first eruption of secondary yaws, suffered from relapses with similar lesions. Probably relapses of typical generalized secondary lesions do not usually occur later than 2 to 3 years after the first eruption, but secondary lesions about the lips (Fig. 9) or on the soles (Fig. 10) may recur after many years.

In a series of cases radiographed were many who had bone lesions but at the time had no yaws skin lesions. In the radiographs of some of these there were active bone changes similar to those in cases with tertiary skin lesions. These were included in the tertiary group and in some of them tertiary skin lesions subsequently developed. The changes in the radiographs of the remaining cases were comparable with those seen in the cases in the secondary group. The reappearance of secondary skin lesions in some of these confirmed their secondary character.

The occurrence of tertiary lesions is the only evidence of passage out of the secondary stage, except the finding of a negative serum Kahn, which would indicate that the disease has ceased. Patients who have had secondary yaws lesions, whose sera are Kahn positive, and who, at the time, have neither secondary nor tertiary lesions are to be regarded as falling into a latent stage, either secondary or tertiary. Some of these patients may attend clinics complaining of pain in the bones.

#### THE CLINICAL LESIONS OF YAWS.

##### THE INITIAL AND SECONDARY STAGES

Of 152 secondary cases with typical secondary skin lesions that were radiographed because of bone lesions 69 per cent. (105) were 5 years of age or less 86 per cent. (131) 10 years or less and only 10 per cent. (fifteen) were over

15 years. The youngest case was 18 months and the oldest 40 years of age. Fifty-three per cent were females. Yaws infection during the first year of life is very unusual.

The initial lesion was present in 26 per cent (forty), and its scar was recognized in 40 per cent (sixty) of cases. In eight of these 100 cases the initial lesion was said to have originated in a previously existing ulcer.

In half of the cases the generalized secondary lesions were associated with a relapse, but the number of secondary relapses occurring in any one case depended upon the duration of the infection. The number of skin lesions was not necessarily greatest during the first eruption. Successive eruptions often appeared before preceding ones had healed, so that lesions in various stages of evolution were sometimes present at the same time. In late relapses the lesions were most numerous about the lips, axillae, genitalia and anus, but these lesions resembled yaws not condylomata lata. This resemblance was also observed in early secondary yaws skin lesions in adults. Itching often accompanies these secondary lesions especially when they are scabbed.

#### *Atypical Secondary Skin Lesions*

Various atypical secondary skin lesions were observed, usually in association with typical lesions. These consisted of forms which, compared with the fully developed papilloma, appeared abortive. They included (a) pigmented macules (Fig 11) with slightly desquamating margins, (b) localized, sometimes serpiginous areas of fine desquamation (Fig 12), (c) small papules, often in groups (Fig 13), and (d) small, often slightly umbilicated, hyperkeratotic papules (Fig 14) on the skin of the knee just below the patella. The Lango recognize all these lesions as the "spittle" or "thorns" of yaws. These include the "roseole pianique" and the "pianides" of MONTEL (1944).

Some of these lesions may develop after the earlier typical secondary eruption has healed. No case of generalized desquamation was observed and often localized desquamation was only apparent after the skin had been rubbed or stretched.

#### *Other Secondary Lesions*

Onychia was infrequent. Dorsal ganglion (Fig 15) about the wrist was seen in six cases and swelling of the knees (Fig 16) with free fluid in the joint cavity in seven, both lesions often cleared up spontaneously. Secondary lesions in the buccal mucous membrane (Fig 17) were observed in nine of these 152 cases. The microscopical appearance of these lesions is identical with that of typical secondary skin lesions (HACKETT, 1939).

Bone lesions are frequent during the secondary stage and are not destructive. The findings of MAUL (1918) that 20 per cent of cases of yaws develop bone lesions, would probably be conservative for Lango for all stages of yaws. The bone lesions are often multiple and are usually indicated by pain, swelling and tenderness. "Rheumatic" bone pain, even in the absence of recognizable



bone lesions is experienced at some time by most cases of secondary yaws. The swollen fingers of polydactylitis (Fig. 18) were recognized by the Lango as "yaws" even in the absence of skin lesions.

The characteristic radiographic appearances of secondary bone lesions are focal rarefactions (rarefying osteitis) and periosteal deposits (periostitis) (Fig. 19). One or other at times appears to be the earlier change, but most often such distinction is impossible. These bone lesions involve the whole shaft of the bone, or a large part of it, rather than small and localized areas. The development of these lesions is rapid and spontaneous resolution, which is hastened by specific therapy usual in a few weeks or months. Epiphyseal changes such as occur in rickets or congenital syphilis were not observed.

On healing all evidence of the rarefactions usually goes, but some of the periosteal deposits may become organized to the surface of the bone and lead to cortical expansion and bony thickening (Fig. 20). Sometimes the bones return to nearly normal appearance. Relapses may occur. The characters of secondary bone lesions, i.e., extensive changes, many bones involved and absence of destruction, are well summarized in the polydactylitis (Figs. 21 and 22).

No gross goundou was seen in Lango such as illustrated by BOTREAU ROUSSEL (1925). Among the 152 secondary cases with skin lesions, minor degrees of goundou (Fig. 23) were observed in 15 per cent. (twenty three). In many of these when seen later the swellings had decreased or disappeared. Among the secondary cases no lesions of the skull or ribs were observed. No bone lesions ulcerated through the skin. There were no spontaneous fractures nor any radiographical evidence of joint lesions. Sabre tibia was frequently seen, though it, probably only in part resulted from yaws.

The epitrochlear glands and spleen were enlarged in nearly 90 per cent. of the cases examined but the splenic enlargement was almost certainly due to malaria. The lymph gland enlargement, which is so frequent in secondary yaws, may in part depend on infection of the numerous breaches of skin surface, usually present in African peasants. The pupil and knee reflexes were active and the heart normal in 90 per cent. of cases. In the other 10 per cent. either the knee-jerks were not elicited or systolic cardiac murmurs were present.

Of sixty three secondary cases with bone lesions, but with no typical skin lesions, 68 per cent. were 10 years of age or less and 17 per cent. were over 15 years of age. 54 per cent. were females. The scars of secondary skin lesions were observed in 80 per cent. and the epitrochlear glands and spleen were enlarged in 90 per cent. In about 10 per cent. of cases the knee jerks were not elicited. The heart and pupils were normal in all cases. Atypical secondary skin lesions were present in six cases, goundou in ten, dorsal ganglion of the wrist in six and free fluid in the knee joint in three.

Lesions of the palms and soles frequently occur as part of the generalized secondary eruption and resemble the relapse lesions (Fig. 10). However a



Fig 1 —Initial lesion

Fig 2 —Typical secondary skin lesions

Fig 3 —Erosive secondary skin lesions on lower limb of sick child

Fig 4 —Scars of secondary skin lesions in right axilla

Fig 5 —Superficial spreading tertiary skin lesion of right breast

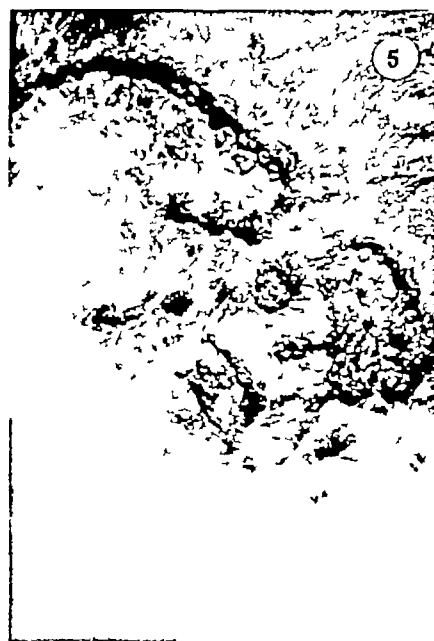




Fig. 6 —Tertiary subcutaneous nodules which gives rise to indolent keratosis, on left foot.

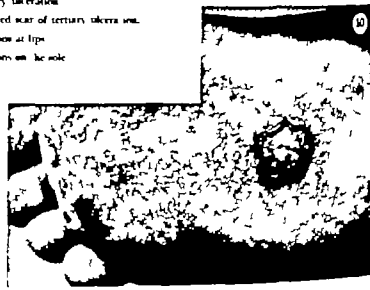
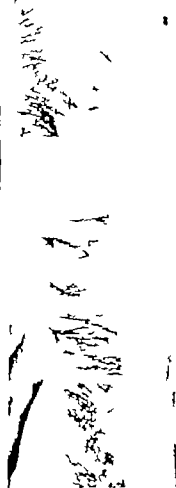


Fig. 7 —Localized indolent tertiary ulceration

Fig. 8 —Atrophic, deeply pigmented scar of tertiary ulceration.

Fig. 9 —Relapse of secondary lesions at lips

Fig. 10 —Relapse of secondary lesions on the sole



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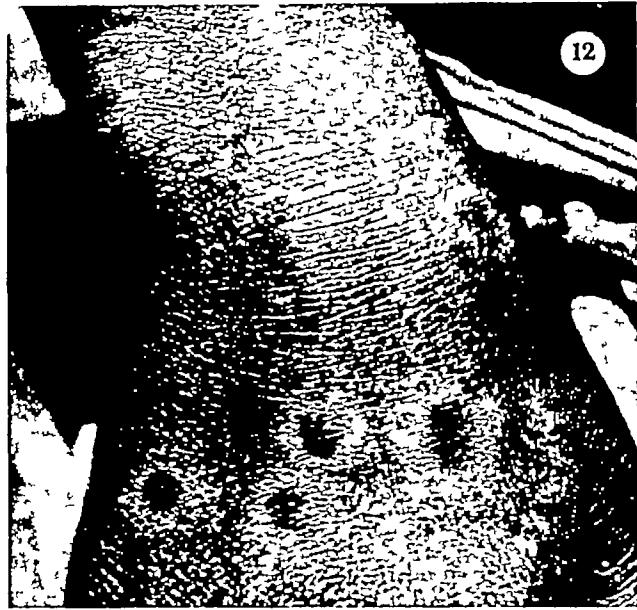


Fig 11 —Localized secondary desquamation on outer surface of right upper arm

Fig 12 —Pigmented secondary macules with desquamating margins on dorsum of right hand

Fig 13 —Small secondary papules often groups on lower thoracic area posteriorly

Fig 14 —Small, often slightly umbilicated hyperkeratotic secondary papules on left knee

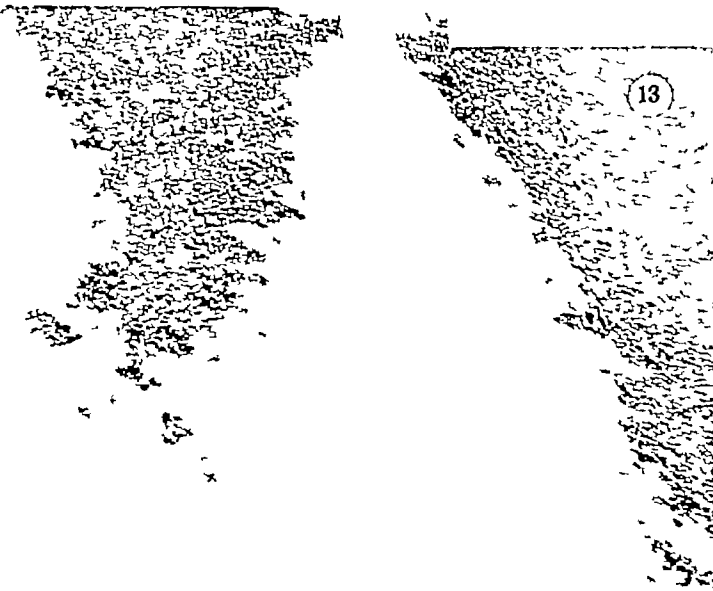




Fig. 15 —Dorsal ganglion of left wrist



Fig. 16.—II distarthrosis of left knee

Fig. 17 —Secondary lesions on basal nasocorn metastasis



Fig. 18 —Dactylitis and swelling of forearm bones (secondary)





Fig 19 —Characteristic secondary bone lesions

Fig. 20 —Same limb as Fig 19 11 months later nearly complete resolution

Fig 21 —Secondary polydactylitis

Fig 22 —Same hand as Fig 21 11 months later nearly normal appearance

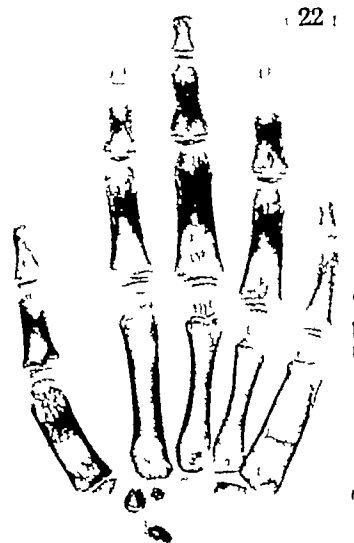




Fig. 23 — Gourdon



Fig. 24 — Non-gra ulcers on secondary palmar lesions

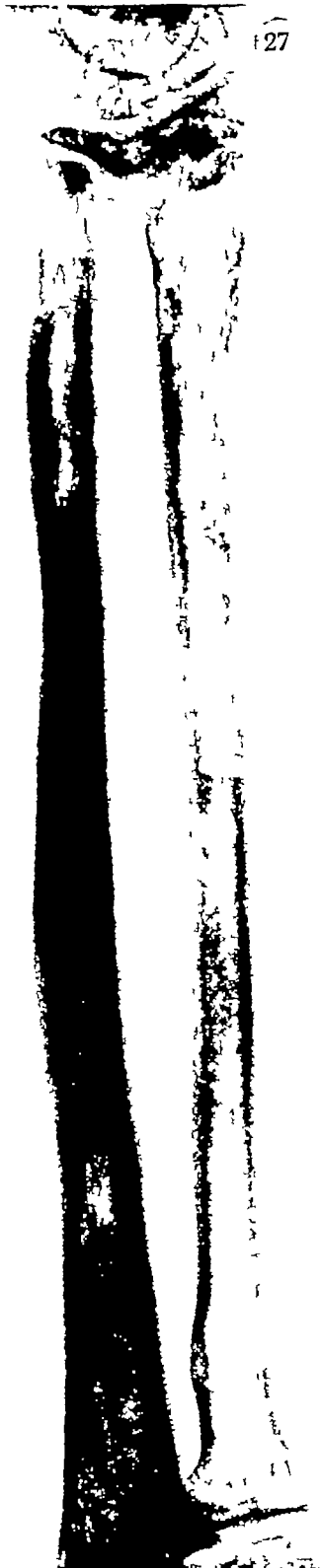
Fig. 25 — Non-granulomatous secondary palmar lesions



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27



(28)



Fig 26 —Characteristic destructive tertiary bone lesions

Fig 27 —Same limb as Fig 26 6 months later final cortical thickening and bone expansion

Fig 28 —Tertiary arthritis

Fig 29 —Same hand as Fig 28 5 1/2 months later residual changes

29







Fig. 30.—Gangosa (tertiary).

Fig. 31—Palatal ulceration and perforation, an early stage of gangosa (tertiary).

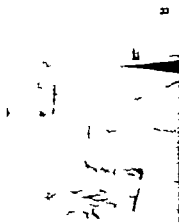


Fig. 32.—Joints—ulcer nodules at elbow (tertiary).

Fig. 33—Pre patella bursal enlargement (tertiary).

Fig. 34—Tertiary palmar lesions.

Fig. 35—Tertiary plantar lesions.



large range of appearances results from thickening and desquamation of the palms and soles (Figs 24 and 25). In some cases much of the palm or sole may be involved accompanied by pain and reduction of capacity for agricultural work. Spontaneous resolution is often slow, but is hastened by specific therapy and usually results in return to normal.

#### THE TERTIARY STAGE

Of 119 tertiary cases radiographed for bone lesions, 20 per cent were 10 years of age or less and 60 per cent were over 15 years of age. The youngest case was aged 5 years, 60 per cent were females. The scars of secondary skin lesions were observed in 80 per cent. The epitrochlear glands were enlarged in 70 per cent and the spleen in 55 per cent. Apart from cases with gangosa, no active lesions of the buccal mucous membrane were observed. In about 10 per cent of cases the knee-jerks were not elicited, but the pupils invariably reacted to light and accommodation. In a few cases systolic cardiac murmurs were present.

In about a quarter of the cases there were tertiary skin lesions and in about the same number bone lesions had ulcerated through the skin. In a fifth of the cases scars of tertiary ulceration were seen. Tertiary palmar or plantar changes were present in four cases. Contractures of the fingers were present in four, ganglion in two, and free fluid in the knee joints in five. The Dupuytren-like contractures of the fingers were often unassociated with any obvious palmar changes and sometimes occurred in subjects who had, apparently, never undertaken heavy manual work. It is highly probable that these are not due to yaws, but that their incidence in tertiary cases is related to the higher age group of those cases.

Bone lesions are frequent during the tertiary stage and are destructive. MAUL's (1918) figures include some tertiary lesions. Pain is present. However, since only one or a few localized lesions (nodes) usually occur, pain is not as pronounced as in secondary cases. Single bones rather than many bones in the hands or feet are affected, and carpal and tarsal bones may be involved.

The characteristic radiographic appearances of tertiary bone lesions (Fig 26) are well defined cortical rarefactions, usually localized and often containing debris ("gummata"). Localized periosteal deposits may sometimes develop on the related cortical surface, and into these the rarefaction may extend. Finally, the lesions may ulcerate through the skin. Occasionally diffuse and extensive rarefactions with periosteal deposits (osteo-periostitis) are seen. Development of all these lesions is relatively slow and spontaneous resolution is not so marked, probably taking months or even years. Response to specific therapy, too, is slower, and in either instance some changes usually remain in the bone such as cortical thickening or increased cortical density (Fig 27). Relapses may occur. The bone lesions of the hand summarize the localized changes, the few bones and the destruction of the tertiary bone lesions (Figs

28 and 29). Published descriptions of yaws bone lesions do not clearly differentiate secondary from tertiary lesions (HACKETT 1948b).

There were nodes on the skull in twenty cases, goundou in four and gangosa in three. Nodes on the skull and tertiary ulceration occurred at all ages, but were much more frequent in older cases. Although only three cases of gangosa were present among those radiographed, several other cases (Fig. 30) were seen. Gangosa and secondary skin lesions were never seen in the same case at the same time. Thus, together with its destructive character and association with other tertiary lesions, leaves little doubt that gangosa belongs to the tertiary stage. It often appears to start in the hard palate and a minimal lesion is a palatal perforation (Fig. 31).

The clavicles were thickened in twenty six cases, the ribs in six cases, the scapulae in five and the sternum in one case.

Spontaneous fracture of a long bone was observed once and gross active articular changes in a large joint twice. These lesions were not frequent among the community. Disorganization of phalanges resulting in deformity or reduction in length were seen in several cases. Bony ankylosis was seen only once, although in a few cases contractures in scars from tertiary ulceration had caused limitation of joint movement. Many authors speak of spontaneous fractures, arthritis and ankylosis as if they were frequent.

Patchy depigmentation of the skin, without preceding ulceration, was observed in a few cases. Its relation to pinta (PARDO-CASTELLO and FERRER, 1942; SMITH 1930) is undetermined. Juxta articular nodules (Fig. 32) and pre patellar bursal enlargement (Fig. 33) were each observed only once among the cases radiographed. These lesions were more frequently seen in older people in the community and almost certainly belong to the tertiary stage.

Lesions of the palms and soles frequently occur during the tertiary stage. They are often extensive and are characterized by thickening and erosion (Fig. 34). These changes are often difficult to differentiate from those in the secondary stage, but the progress and response to treatment are slower and the final result is more often a depigmented atrophic skin (Fig. 35). Published descriptions of palmar and plantar yaws lesions do not clearly differentiate secondary from tertiary lesions (BAERNANN 1911; HALLENBERGER, 1916; GUTIERREZ, 1923; SMITH 1930; HERMANS, 1931 and 1939; SIMPSON 1933; MONTEL, 1944). Tertiary palmar and plantar changes appear to be more frequent in cases without than in those with bone lesions.

The above descriptions indicate the variety of lesions observed in Lago. From published descriptions a similar variety apparently occurs in other communities where yaws is prevalent.

#### THE COURSE OF YAWS.

Some authors speak of initial and secondary lesions that persist and later undergo the destructive changes of the tertiary stage. There was no evidence of this nor of the occurrence of tertiary lesions in the scars of secondary lesions

in the cases studied. Accidental auto-infection is not a necessary explanation of the occurrence of secondary lesions on the buccal mucous membrane, since the distribution of the skin lesions indicates that spirochaetes must be circulating in the blood.

It is possible that some of the subsequent eruptions of secondary lesions may be accidental re-infections. Although experimental re-infection has been shown possible for 2 to 3 years after infection (TURNER, 1936), it is probable that the degree of infection necessary to produce this would rarely be encountered under natural conditions. Auto-inoculation by secondary lesions may occur in the axilla, under the breast and between the thighs and buttocks. Here friction, moisture and duration of contact play important parts.

Secondary lesions of characteristic yaws appearance on the lips, palms or soles were seen in a few older children and adults who were said to have had the generalized eruption in childhood, and to have had no further skin lesions until these appeared. It is probable that these relapsing lesions help to maintain the infection in a community. The late recurrence of such secondary lesions indicates the difficulty of defining the secondary and tertiary stages in terms of duration of infection.

Although it is not known precisely what percentage of yaws cases fail to develop an initial lesion at the site of infection, a very large proportion, within a few months of infection, develop typical secondary skin lesions. It is possible that neither initial nor typical secondary skin lesions may appear and the patient may be unaware that anything is amiss until secondary bone, atypical secondary skin lesions or tertiary lesions appear.

Most of the more typical cases, after 2 to 3 years, will become free from secondary skin lesions, although some may have active secondary bone lesions. Some cases after the healing of the secondary eruption probably overcome the infection completely, and their sera then give negative Wassermann and Kahn reactions. Of those which remain infected (latent secondary stage) many develop no further frank lesions, but suffer indefinite aches and pains of varying severity, for which, although yaws may be in part responsible, there are usually other possible causes. As time goes on an increasing number of these may overcome the infection.

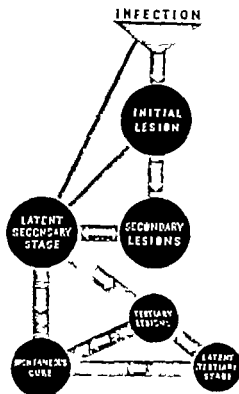
Those cases that do not follow this benign course undergo a change in the reaction of their tissues and develop destructive lesions of the tertiary stage. The time at which this change occurs in relation to the duration of infection is indefinite.

The youngest of the 119 patients with tertiary bone lesions was 5 years of age. Of the twenty-four who were 10 years of age or under, excluding one aged 10 who could not recall any earlier lesions, all except four said they had had the disease for some years. These four were aged 5 to 6 years, two were said to have had yaws for 1 year, and the others for 3 and 5 months. This information regarding time from patients' statements must be accepted with caution. It is probable, however, that tertiary lesions may develop in some

cases, especially in children, as early as 2 or 3 years after infection. Of the remaining ninety-five tertiary cases over 10 years of age, all except three gave histories of having had yaws in childhood, *i.e.*, some years previously.

Statements in the literature (*e.g.*, STANNUS 1939) that 50 per cent. of bone lesions in yaws occur within 12 months of the initial lesion, and that in any group of yaws cases, 50 per cent. of those with bone lesions will be under 15

DIAGRAM



### POSSIBLE COURSES OF YAWS

years and 75 per cent. under 20 years, are apparently based on the findings of the twenty cases of MAUL (1918).

After the resolution of tertiary lesions spontaneous cure may occur or a latent tertiary stage may be entered. From the latter tertiary lesions may relapse or the infection may be overcome.

### SUMMARY

The initial lesion may heal before secondary ones appear or may persist into the secondary stage. All other active lesions, apart from a few such as

ganglion, hydrarthrosis and goundou, which may occur in more than one stage, are either secondary or tertiary. In many cases secondary lesions cease to relapse 2 to 3 years after infection. In some cases lesions of the secondary type may develop years after the earliest manifestations of infection have healed, while in other cases tertiary lesions may appear within a few years of infection. In many cases there is a more or less symptom-free period of years between the healing of the secondary eruption and the development of tertiary lesions. Lesions of these two types are never observed in the same patient at the same time, nor is the initial lesion present when tertiary lesions have developed. Brief descriptions, with illustrations, are given of the chief manifestations of yaws seen in Lango. Diagram 2 indicates the possible courses of yaws.

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A short film made by Dr HACKETT was shown which stressed the main lesions of the disease as seen in Uganda.

## DISCUSSION.

**The President** We are very much indebted to Dr HACKETT for his very lucid account of his investigations on the course of yaws. As he has told me on many occasions, there has been in the literature a good deal of confusion between the secondary and tertiary lesions in yaws. Dr HACKETT has shown us, in the first place, the primary lesions, the typical secondary lesions and the typical tertiary lesions. He has then described and illustrated the less typical lesions which are associated with the typical secondary and tertiary lesions. These he has concluded are secondary or tertiary lesions as the case may be. I think there can be no doubt that he has given us a clearer picture of the actual course of yaws in its development from one stage to another. Many here know a great deal more about yaws than I do and I hope everybody who wants to speak will do so. Dr STANNUS has had a great deal of experience of yaws in Africa and I call upon him to open the discussion.

**Dr H. S. Stannus** I am sure I am expressing the feelings of everyone when I say how much we have enjoyed Dr HACKETT's paper this evening. I was very pleased to see that yaws was to be the subject of his address as I think too often we neglect some of our old friends. Dr HACKETT has touched on many points of interest which will lead I am sure to a good discussion. May I briefly refer to some of them.

Has he any explanation as to why the unitarian view of treponematosi seems to find favour in the United States, while in Europe we regard yaws and syphilis as separate entities? I have followed HUDSON's views from the beginning, views based on his observations among the Arabs in the Euphrates valley. I saw his photographs and discussed the condition called "bejel" with him, yet I was left in no doubt in my own mind that the condition was syphilis and not some bastard type of yaws.

Can Dr HACKETT offer any explanation of the difference in incidence of yaws and syphilis at Lara and at Masaka? It would appear to be a matter of some importance.

Reference was made to "the confusion in some recently published clinical descriptions" of the disease. May we be told where these descriptions may be found, and wherein they are confused?

His description of the clinical aspects of the disease does not differ in any material point from the picture with which one was familiar (alas!) many years ago. I wonder whether Dr HACKETT and others have any views on lesions of the mucous membranes? He notes in nine of the 152 cases lesions of the buccal mucosa were they lesions which had "crept" over the mucocutaneous junction? I myself have never seen a lesion which was not susceptible of this explanation. The patchy depigmentation of the skin, with its special distribution, again is an interesting phenomenon. One saw many such cases but what is their pathogeny? They do not to my mind resemble the condition

seen in pinta—another separate treponematosi. He saw no case in which an old and persisting primary yaws took on the characters of a tertiary lesion. It would be of interest if other speakers would give their observations. I believe the change does take place. In regard to lymphatic glands, I believe only a very general statement can be made. A generalized adenopathy may occur in yaws as in syphilis.

I had rather hoped Dr HACKETT would have finally thrown overboard the old nomenclature of primary, secondary and tertiary stages and given us one based on sound pathological findings. Would Dr HACKETT tell us why he regards yaws as only one factor in the aetiology of sabre tibia? He has always been particularly interested in the bone lesions. Can he describe to us the finer pathological changes underlying the gross changes depicted in a radiogram. Wherein pathologically do the periosteal deposits in early bone lesions differ from periosteal deposits in later stages?

Lastly, Dr HACKETT has referred to goundou and to BOTREAU-ROUSSEL. The occurrence of the truly remarkable series of cases of goundou and framboesial osteitis collected by that author has never been explained. I wonder whether Dr HACKETT can suggest any explanation of the uneven incidence of this condition? Is some nutritional deficiency a possible factor?

ROUSSEL points out the close resemblance between goundou and leontiasis ossea. Many years ago I collected all the published cases of leontiasis ossea and other similar conditions.

I found I could match practically each of ROUSSEL's cases with one from my series—leontiasis ossea, the "creeping periostitis" of LAWFORD KNAGGS, fibrocystic disease of bone or Paget's disease.

[Slides were shown by Dr STANNUS to illustrate some of these points.]

**Dr G M Findlay** As a comparison with the account of yaws in East Africa, a few remarks on the same disease in West Africa may not be without interest, as in West Africa the population consists largely of Sudanic negroes and Hamites, with semi-Bantu and Bantu tribes only in South-Eastern Nigeria.

During the war, the immensity of the yaws problem in the British West African colonies became fully apparent. Apart from insufficient physical development, with failure to reach a height of 5 ft 2 in. and a chest of 32 in., yaws was the most important single cause for the rejection of recruits. Of 4,647 recruits examined in the Northern Territories and Northern Ashanti in the autumn of 1942, no fewer than 3,260 or 70.1 per cent. were rejected. In Ogoja and Okigwi in Nigeria 75 per cent. were rejected, chiefly for yaws—crab yaws of the soles of the feet, chronic bone lesions, ulcers and tissue paper skin on the legs. The rejection rate for recruits throughout British West Africa was never under 50 per cent. and in some areas of Sierra Leone and Southern Nigeria reached 90 per cent. Similarly, yaws was a major cause of invaliding from the Army. This state of affairs is not surprising when the extent of yaws



in the civil population before the war is taken into account. In Nigeria, in 1936 and 1937 110,588 and 90,225 patients were treated in Government hospitals and dispensaries. As the population of Nigeria is approximately 21 000,000, this means that in each of these 2 years just under one in 200 of the whole population was treated for yaws. A reliable observer among the Tiv of the Benue Valley has estimated that 85 per cent. of the population has yaws in childhood, and the other 5 per cent. when they are grown up. In Zairungu Dispensary in the Northern Territories of the Gold Coast, about 300 patients are treated every month and 50 per cent. of these have yaws incidentally the social definition of a dispensary in the Northern Territories is a place where you get treatment for yaws and worms. In the Northern Territories, with an estimated population of between 750 000 to 800,000 the yaws cases treated in 1936 were 31,277 and in 1937 47 104 in other words approximately one in twenty of the population received treatment in each of these 2 years. In 1937 in Eastern Dagomba, 14 000 out of an estimated population of 80 000 received yaws treatment.

From the Gambia to the Cameroons the picture is very much the same even in those areas where yaws campaigns have taken place. Only in Northern Nigeria is yaws relatively uncommon and here syphilis takes its place—cases of cardiovascular disease and of neurosyphilis were far more common in soldiers hailing from Northern Nigeria than from any other area. In Northern Nigeria, also it is occasionally possible to encounter a tribe such as the Iregwe who live round Miango Mountain on the Jos Plateau which has no word for yaws or syphilis. In all other areas yaws is so well known that it is one of the few diseases which is unassociated with ideas of witchcraft or magic as regards either causation or treatment. Only among the Twi and Gã, when chronic yaws ulcers refuse to heal, is witchcraft considered, while among the Tiv gangosa is thought to be due either to a malignant spirit, the *akombo a dem*, or to the machinations of the *mbatsar* the local witchgild—then the disease may be fatal.

Throughout West Africa yaws is primarily a disease of the bush rather than of the large towns. In Abeokuta and Ibadan it is not common to see primary or secondary yaws although there is plenty of tertiary yaws. Yaws is also a seasonal disease—investigations in villages show that during the rains there are then more cases of primary and secondary yaws than at any other time.

In most communities the primary yaw is rare before the age of 18 months the peak years are from 2 to 5 with a gradual decline till puberty. Among women in the early twenties there is again a rise in incidence. Of eight young women with primary or secondary yaws the primary was in every case on the breast or chest, the infection having been caught from a yaws infected child who was still breast fed.

Some light is thrown on the method of infection by a study of the site of the primary yaw. In 100 consecutive primary cases seen in the Gold Coast Colony, the primary yaw was situated as follows —

Buttocks	23	} 57	Ankle	6
Perineum	22		Knee	5
Thighs	12		Axilla	4
			Shoulder	3
Nose	5	} 16	Arm	5
Lips	5		Wrist	1
Chin	4		Dorsum of hand	1
Nasal septum	1		Finger	1
Eyelid	1		Back	1

Thus the parts of the body in contact with the ground when the patient is sitting are the areas most commonly affected. The clothed children of clerks and literate Africans are much less likely to become infected.

It is questionable how far flies contribute to the spread of yaws in West Africa.

Many mothers in West Africa deliberately expose their children to infection in the belief that primary yaws in childhood is less dangerous than in adult life. In fact, the Tiv believe that if an adult contracts yaws he will never get properly right. Some tribes, such as the Dagomba, think that if treatment is begun within 6 months from the date of the appearance of the primary yaw the disease will inevitably recur. Thus it is found that the mass of patients coming to hospital are those with chronic ulcers, bone lesions and yaws feet. Of 5,136 cases treated in Tamale Hospital 75 per cent were tertiary.

A point which is not often emphasized is the frequency of overt signs of food deficiency in children with secondary and early tertiary yaws. As an example, one of many surveys may be quoted. In a Dagarti village, Takpo, some 15 miles west of Wa, at the end of the dry season in May, 1945, it was found that there were in the whole village 290 children under puberty. The children were for the most part well nourished as large amounts of *pito*, made from fermented guineacorn, were drunk. Of 233 without signs of yaws nine, or 3.8 per cent, had evidence of deficiency disease (glossitis, cheilosis, angular stomatitis, scruffy scrotum or crazy-pavement skin). Of forty-seven children with primary or secondary yaws, seven, or 14.8 per cent, had very marked signs of food deficiency. Dysentery and malaria are known to precipitate signs of food deficiency.

Pain and aching in one limb followed by pain in the knees and then in the other joints are the most common premonitory symptoms of yaws.

With regard to the secondary lesions, the frequency of circinate eruptions may be mentioned. Where a secondary eruption recurs after it has disappeared,

following insufficient treatment, the lesions quite frequently do not recur at the old sites but in fresh areas. The occurrence of a secondary lesion on the glans penis is not uncommon. Cases where the primary yaw still persists in association with a well-developed secondary eruptions are sometimes seen more rarely a sabre tibia may be found in association with a still persisting secondary rash. Long persistence of a secondary yaw is well known to many tribes. The occurrence of a semi-persistent yaw on the buttocks among the Fiv renders the youth an object of ridicule and prevents his obtaining a wife.

The occurrence of a meningismus during the secondary stage, accompanied by an increase in small lymphocytes in the cerebrospinal fluid, should not be forgotten the increased cell count tends to disappear in 2 to 3 months but further observation is required.

It is doubtful whether any hard and fast line can be drawn between late secondary and early tertiary bone lesions. In West Africa gummatous osteitis with periostitis comes on much more rapidly and acutely than it does in East Africa pain is severe and sometimes there is fever. The tibia is most commonly affected but other sites are the lower end of the radius, the inner end of the clavicle and the elbow region. Dactylitis which may be a secondary change, is more common in the fingers than in the toes. Trauma, even a bruise, may be followed by a yaws periostitis. The so-called big heel is probably a yaws periostitis or an inflamed bursa over the tendo Achilles. Ulceration is most common where bones lie near the skin recurrence is frequent and when the skin heals it may be puckered and of tissue paper consistency. Depigmentation is very common while contractures of the fingers or toes are by no means rare.

It is curious that yaws ulcers, though smears invariably show staphylococci, rarely become infected with Vincent's spirochaetes and fusiforms. The infectivity of these ulcers requires further study.

Crab yaws with hyperkeratosis is most common in the rains and follows trauma. It may affect one or both feet while in rare cases the palms of the hands may be involved. In one instance the flexor surfaces of the toes were involved but the soles were unaffected. In the 51st West African Division, during the return from the Kaladan in 1944 the soldiers found their boots too heavy and threw them away a veritable epidemic of yaws feet occurred. Yaws feet have to be distinguished from self inflicted injuries made with a razor blade, from cracked feet, more common in the harmattan, from tiny pitting over the heel and heads of the metatarsals, changes which have been seen in Europeans, and more rarely from rat bites and from the bites of the caterpillar of the tiger beetle known in Northern Nigeria as the *feru* or *feru*. There is some evidence that this beetle may live as far south as Enugu.

Ganglion was very common in soldiers gonorrhoea and gangosa were, of course, rare. Among 80,477 African soldiers admitted to hospital there were sixty-eight cases of ganglion, two of which were on the dorsum of the foot.



**Dr C C Chesterman** I am gratified to hear that Dr HACKETT has the binocular view of the question, which seems to me definitely the right one in my experience of 25,000 cases. One wonders whether the persistent secondary yaws do not really prevent the tertiary lesion. I never found anyone with open crab yaws on the feet who had any tertiary lesion at all. It may be that they form a fixation point or produce a sort of auto-vaccination preventing the lesion developing. I can confirm the hesitation of tribes people to having their children treated until the really florid eruption appears. One got the impression that cases that were treated when only a few secondaries were visible had relapses more often than those that had treatment when the florid eruptions were out. I wonder whether the relative humidity is not the explanation of the differences of the types of lesion described. They are more florid in the moister regions of the body and of the world. The capriciousness of the onset of the tertiary lesions has led some primitive peoples to attribute this to witchcraft, and it is supposed to be the curse from the neglect of a certain taboo. One often saw tertiary lesions appearing in women after childbirth. For the treatment of the tertiary lesions I have much more confidence in iodides than in bismuth or arsenic. I collected a series of cases of melanotic carcinoma in association with crab yaws, it is one of the commonest malignant tumours in African negroes. Has Dr HACKETT any evidence of perivascular round celled infiltration around the tertiary lesions of the skin? It is supposed to be the criterion between yaws and syphilis.

**Professor T F Hower** At the risk of being tedious, I should like to make a few remarks in favour of the unitarian view. I spent a long time when I was in the Sudan trying to collect a series of cases of yaws in the southern part of the country for comparison in various respects with patients suffering from syphilis in the north. I had studied the table of differential diagnosis in MANSION BAHR's book and was firmly convinced that it should be possible to make a diagnosis of yaws. Unfortunately although I visited the principal yaws areas in the south, I found it quite impossible to distinguish between yaws and the more florid examples of syphilis occurring farther north. In fact there was a gradual gradation between the types encountered in the south and those in the north where the disease, especially in Khartoum, was frankly venereal syphilis. I examined the cerebrospinal fluid in a great many cases and found a moderate pleocytosis in many of the classical yaws cases but no neurological signs. As one travelled north neurological signs became manifest and frank meningo-vascular syphilis was encountered commonly in Khartoum. The chief factor of difference seemed to be the race of the people involved, although there were also climatic differences. I would suggest to Dr FINDLAY that his natives from the north who contracted neuro-syphilis did so because they were a different race from those in other areas who had yaws without clinical neurological signs. In the Sudan I collected biopsy specimens from

lesions of yaws and comparable stages of syphilis, from different parts of the country, and found it quite impossible to discover any histological difference between them. Dr HACKETT mentioned that a lesion of the soft palate he excised for biopsy showed the histological characteristics of yaws in the secondary stage, but I should very much like to know why it was not characteristic of syphilis. I do not believe that histological investigations will help us in the least to differentiate between the two conditions. A more helpful method, in my opinion, is the collection of strains of spirochaetes and study of their action on experimental animals. This has already been done to some extent but the findings are inconclusive. Workers in Jamaica claimed that the testicular lesions in rabbits with yaws were characterized by a granularity of the tunica albuginea, but I saw some of these specimens and the change was by no means constant. I collected a number of strains of spirochaetes myself in the Sudan from different kinds of cases and injected them into rabbits and was unable to discover any difference between them, although some were from yaws in the south and others from typical syphilis in the north. My work was not completed because I left the country too soon.

I should like to deprecate a too dogmatic division of yaws from syphilis since I am certain the criteria do not warrant it. I should very much prefer to call the condition "tropical syphilis". Finally, I very much hope someone may study the strains of spirochaetes by animal inoculation since I believe this is the most hopeful line of research.

**Dr F Murgatroyd** It has been suggested that the treatment of yaws is less efficacious in the early stages than it is when the disease had been allowed to run its natural course for some time, and I understood Dr CHESTERMAN to say that he found that patients with minimal lesions responded less favourably to treatment than did those with more florid lesions. I wonder, however, whether the two classes of patients received the same degree of treatment, or whether the difference in the results of their treatment might not be explained by the natural tendency of patients with minimal lesions to leave off treatment sooner than did those whose lesions were more pronounced.

**Dr Chesterman** (answering Dr MURGATROYD's question) With a view to ensuring complete treatment, we used to insist on payment in advance, or a money pledge to be returned after finishing the cure.

**Dr J F E Bloss** I should like to ask Dr HACKETT for further information *re* the nutritional side of the disease. In the Anglo-Egyptian Sudan the disease is undoubtedly far more common among the pastoral Nilotic tribes than among the agrarian Zande. The former can be regarded as "blood and milk" eaters, while the latter are purely vegetarian. The difference in incidence between two such widely different groups of tribes would suggest that nutritional differences might have some effect on the incidence of the disease.

Dr R B Leech Was any evidence found such as the prevalence of tertiary lesions that yaws used to be more common at Masaka suggesting the development of some tribal immunity?

Dr Hackett in reply to Dr STANNUS's question why America was such a stronghold of the belief that yaws and syphilis were the same disease—it is possible that it was one way of refuting the alleged American origin of syphilis which was so strongly contested by certain American writers who were also "uniticists."

Among differences between the populations of Lira and Masaka that may be related to the different incidence of yaws and syphilis were the original pastoral pursuits, strict moral code and undress of the former compared with the agricultural activity, free moral code and clothing of the latter. It is possible that once yaws is well established in a community syphilis may not get established because of cross immunity resulting from childhood yaws.

Recently published confused descriptions of yaws may be found in the last editions of many standard textbooks.

The secondary buccal mucous membrane lesions were remote from any skin junctions and certainly did not arise from any spreading lesion of the skin. Trauma did not appear to play a part. The infrequency of reports of these lesions is possibly associated with the difficulties of examining the buccal mucous membranes where bright light throws dark shadows. A small oral light is essential in these observations.

Depigmentation did not always appear to result from scarring following ulceration.

Yaws was thought to play only a part in the production of sabre tibiae because although many legs were observed for considerable periods, the superposition of tracings of lateral radiographs showed increased curvature resulting only from periosteal deposition and organization on to the anterior tibial cortex. In no case was there any increase in curvature of the posterior tibial outline. In addition, a few young children were found with bowed tibiae in whom there was no clinical or serological evidence of previous yaws.

As regards the presence of spirochaetes in tertiary lesions, they have been found in tertiary bone lesions in a femur brought back from Uganda.

Histopathological differences between yaws and syphilitic lesions have been recognized by HALLENBERGER (1916) in the absence in the former of the classical syphilitic change of the blood vessels. This is not supported by FERRIS and TURNER (1937).

Goundou would appear to be a secondary yaws lesion. Any apparent irregularity in distribution should be checked by adequate observations.

To Dr FINDLAY No evidence of association of typical secondary and tertiary yaws lesions in the same case at the same time was found. Sabre tibiae are more likely to be associated with the generalized changes of the secondary

stage—and probably a major part in their causation is not yaws in origin. No lumbar punctures were performed in the Uganda cases.

To Colonel WATTS. A recent paper by DWINDELLE *et al* (1946) indicates that secondary yaws responds well to penicillin but following total dosages of 1,200,000 O U in 4 days only 10 per cent of cases had negative serum Kahn reactions 6 months later. In a comparable series of cases of syphilis, about 80 per cent of negative Kahns would be expected.

To Dr CHESTERMAN. No evidence was seen of secondary eruptions protecting against tertiary lesions, although SCHOEHL (1928) observed this in his experiments with monkeys in Manila.

Regarding the relation of type of lesion and relative humidity, nothing remarkable was observed in the Lango. They said yaws was more prevalent in the wet season. There were peaks of yaws attendance at certain seasons but this was more probably related to slack periods of agricultural activity. No incidence of tertiary lesions following childbirth or of "melanomata" in plantar lesions were seen.

To Professor HEWER. Although yaws and syphilis may be regarded as two diseases, the tables of differentiation in textbooks purporting to demonstrate this are practically worthless (see STANNUS, 1936). The mucous membrane lesions in the mouth were observed on the hard and soft palates, the anterior pillar of the fauces and once on the tongue. Their histo-pathological resemblance to secondary yaws skin lesions did not imply differentiation from syphilitic lesions. However, macroscopically, their granular surface did resemble yaws rather than syphilitic lesions. It is doubtful if experimental animal infections will declare the difference between yaws and syphilis—such differences are always liable to be discounted on the grounds that they are quantitative and not qualitative. The best approach would be the production of pure cultures of the organisms concerned, perhaps chick-embryo cultures may serve this purpose, then differences in antigenic structure could be sought.

To Dr BLOSS. There was undoubtedly nutritional inadequacy among the Lango, how extensive it was and what part it played in relation to yaws is not known. The Lango diet is mainly, but not entirely, vegetarian.

To Dr LEECH. It would be difficult to obtain data regarding the incidence of tertiary yaws lesions at Masaka since most of the yaws occurred in Banyaruaanda (Belgian Congo) migrant labour who would probably not stay long in the area.

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## INTRODUCTION

Atebrin, first synthesized by METSCHER and MALL, had been extensively used in malaria therapy before the outbreak of war and its value as a polyvalent schizonticide definitely established for the four species of malaria parasites affecting man. Within a short period of the drug becoming available JAMES NICOL and SHUTE (1932) showed that the Rome strain was more sensitive to atebrin than quinine. JAMES (1933) gave 0.3 gramme daily for 6 to 7 days in individuals exposed to three to twenty-one infective bites (*P. falciparum*). No overt attacks occurred in a period of 14 months so that the patients were regarded as being free from malaria. CRUCA and his colleagues (1937) obtained full protection over a period of 12 months in individuals exposed to *P. falciparum* while taking 0.3 gramme of atebrin daily for 11 days. When five individuals were exposed to infective bites (*P. vivax*) by JAMES (1933), while taking 0.3 gramme of atebrin daily for 6 days the primary attack was delayed for from 89 days to 230 or more days.

FIELD (1939) reviewed the experimental findings of different workers in regard to the value of atebrin as a prophylactic in doses of 0.2 to 0.3 gramme daily when given to general paralytics during the incubation period of malaria. He concluded that in *P. falciparum* infection clinical attacks of malaria were completely prevented in a high proportion of individuals while the onset of the infection was delayed several months in a small series of cases of *P. vivax*. At what stage the parasite was attacked whether at the sporozoite stage, early in the incubation period or during schizogony in the later stages of the incubation period was not definitely known, but as small doses continued well into the incubation period were more effective than large doses given only at the time of exposure, the prophylactic action of atebrin was to be regarded as probably schizonticidal, & the medico-curative prophylaxis of SINGER not true causal prophylaxis.

FIELD (1939) also reviewed the results of investigations on natives working on plantations in malarious areas in Malaya while receiving 0.15 gramme of atebrin daily or 0.4 gramme weekly administered in two doses in each of 2 successive days. The conclusions reached were that (1) atebrin in dosage of 0.2 gramme twice a week was appreciably more effective than the daily dose of 0.4 gramme (6 grains) of quinine for suppressing clinical malaria. (2) infection was not prevented, even after 1 year administration, since symptoms returned after drug administration ceased.

Despite the superiority of atebrin in suppressing malaria, FIELD finally concluded that regular daily prophylactic quinine was preferable to twice weekly atebrin owing to the yellow staining of the skin occasional mental disturbances and possible damage to the liver which atebrin was thought to induce. The freedom from harmful by-effects with quinine weighed heavily in its favour. Thus at the outbreak of war in 1939 the status of atebrin as

a suppressive prophylactic drug was uncertain. Even after the fall of Java authoritative opinion in U.S.A. and the United Kingdom was divided in regard to (1) the appropriate dosage of atebirin in non-immunes, (2) its real effectiveness in suppressing malaria under conditions of jungle warfare, (3) the possible toxic effects of prolonged administration. In 1942, as a result of field experience in Africa, HILL (1942) reached the conclusion that 0.4 gramme of atebirin a week was too small a dose for military purposes and advised one tablet (0.1 gramme) daily for troops on active service in areas of *P. falciparum*. Experience indicated that on this atebirin regime recrudescences would be virtually abolished even under strenuous conditions and the malaria problem greatly reduced.

In New Guinea questions were continually being raised by field commanders and staff officers of the Australian Military Forces regarding the efficacy of anti-malaria measures advocated by the Medical Directorate, and whether such prophylactic drugs as quinine (10 grains daily) or atebirin (0.1 gramme on 6 days a week) could control the repeated and heavy malaria infections contracted in jungle warfare. The opinion of regimental medical officers was divided on the question, but at this time the majority regarded chemotherapeutic control as ineffective. One of the major tasks of the L.H.Q. Medical Research Unit established at Cairns was to clarify this difficult situation and decisively answer the question one way or another.

In a previous communication to these TRANSACTIONS dealing with chemotherapeutic suppression and prophylaxis of malaria, the results of experiments on volunteers infected with New Guinea strains of malaria were recorded by FAIRFAX and his colleagues (1945) and the military implications of these experimental findings were discussed. As the present communication is an extension of that work the original conclusions are summarized below.

#### (1) MILITARY IMPLICATIONS OF THE CAIRNS EXPERIMENTS

The original experiments by L.H.Q. Medical Research Unit, Cairns, demonstrated that atebirin in a dosage of 0.1 gramme daily suppressed malignant tertian fever and prevented asexual parasites from attaining microscopic densities in the blood. Furthermore, if the daily dose of atebirin (0.1 gramme) was continued for 28 days after the last exposure to infection, cure invariably resulted. Subinoculation experiments proved the action to be on the asexual blood parasites which appeared in the blood in submicroscopic densities on the 7th day in *P. falciparum*. Provided the blood concentration of atebirin was adequate the blood was completely cleared of parasites in 3 or 4 days. Subsequent subinoculations proved negative and radical cure was effected with all the New Guinea strains of *P. falciparum* tested.

Under similar circumstances atebirin merely suppressed benign tertian malaria. Subinoculation showed that asexual parasites of *P. vivax* first appeared

in the blood on the 9th day in submicroscopic density and that the blood was readily cleared of parasites provided atebryn was present in appropriate concentrations. Despite this result and in contradistinction to the findings in malignant tertian malaria, atebryn administration generally did not result in radical cure for overt vivax malaria developed with great regularity a few weeks after suppressive atebryn administration ceased. This was attributed to the persistence of hypothetical exoerythrocytic (e.e.) forms in vivax malaria and their absence in falciparum malaria.

In multiple intense infections with *P. falciparum* and *P. vivax* suppression was equally effective under a similar atebryn regimen. It was invariably overt B T and never M T malaria which developed when atebryn suppressive treatment ceased.

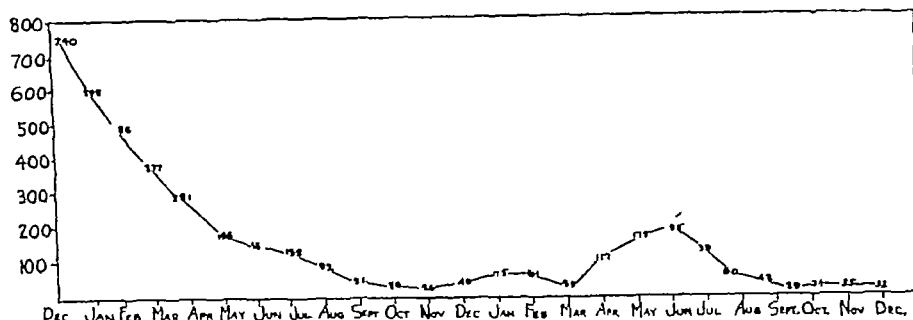
Various factors such as hard physical work, prolonged marching up and down hills, exposure to extreme cold, anoxia, blood loss and injections of adrenalin and insulin failed to produce malaria breakdowns in heavily infected volunteers.

In June, 1944 at the Military Conference on the Prevention of Disease in Tropical Warfare held at Atherton Northern Queensland, under the chairmanship of Lieut. General V. A. H. STURDEE, G. O. C. 1st Australian Army it was officially accepted that with infallible atebryn discipline (1) it would be possible to fight a non immune force for many months in hyperendemic areas of malaria without significant malaria casualties (2) there should be no deaths from malaria, no blackwater fever and no malaria carriers within the force (3) after cessation of atebryn suppressive treatment, the residual problem would be exclusively that of B T malaria. From that time atebryn was taken under stricter supervision and its administration became a disciplinary matter.

Subsequent field experience in New Guinea throughout 1944 confirmed these conclusions, the hospital admission rate falling from 740 per 1,000 per annum in December 1943 to 26 per 1,000 per annum in November 1944 as reported in these TRANSACTIONS (1945). Since that date subsequent experience during jungle fighting largely fulfilled these expectations. A perusal of Chart 1 shows that the incidence of malaria in all Australian troops located in malarious areas in the South-West Pacific, north of Australia, was 39 per 1,000 per annum in March, 1945. Thereafter there was a rapid rise to 185 per 1,000 per annum in June, followed by a fall to 60 per 1,000 per annum in July and 29 per 1,000 per annum in September. The rates for the ensuing months were 34, 33 and 32 per 1,000 per annum. The rise in malaria casualty rates over the April-July period was entirely due to a severe outbreak of malaria in troops fighting at Aitape and Wewak in New Guinea. With this single exception the results of the Cairns experiments were confirmed on a vast scale under field conditions in hyper-endemic areas of malaria in the South-West Pacific throughout 1945.

CHART 1

INCIDENCE OF MALARIA IN AUSTRALIAN TROOPS IN MALARIOUS AREAS NORTH OF AUSTRALIA  
RATE/1,000/YEAR



DECEMBER 1943 TO AUGUST 1945

The rise in malaria incidence from April to July, 1945, was entirely due to a severe outbreak of malaria affecting troops fighting at Aitape and Wewak on the Northern Coast of New Guinea

## (2) MALARIA INCIDENCE IN THE SOLOMONS, NEW BRITAIN AND BORNEO (1944-1945)

The malaria incidence in operational areas other than New Guinea (1944-1945) was insignificant (Chart 2). Out of a total force of approximately 130,000 troops exposed for a period varying from approximately 6 to 12 months, there were 1,256 attacks of malaria. Of these 476 were caused by *P. falciparum*, 658 by *P. vivax* and one by *P. malariae*. In 115 cases, parasites were not found microscopically, the diagnosis being made on clinical grounds. The malaria incidence averaged 0.97 per cent (Table I p 235). In these areas there was one death from malaria and none from blackwater fever.

## (3) MALARIA INCIDENCE IN NEW GUINEA

In New Guinea itself, other than in the Aitape-Wewak area, there were approximately 15,000 troops, a very low malaria incidence was recorded, the monthly rates in 1945 varying from sixteen to fifty-two per 1,000 per annum, being highest in February and lowest in November. In this force deaths from malaria and blackwater fever were nil. The other troops in New Guinea numbered 23,000 and were located in the Aitape-Wewak area.

In the course of this campaign along the northern coast-line of New Guinea extending from Aitape to Wewak, the 6th Australian Division suffered a severe outbreak of malaria—chiefly malignant tertian (Chart 2). To a lesser degree, three Base Sub-area troops were also involved. Infections were widespread with the heaviest incidence in the 16th, 17th and 19th Brigades. The

CHART —  
INCIDENCY OF MALARIA IN AUSTRALIAN TROOPS  
PER 1000 YEAR

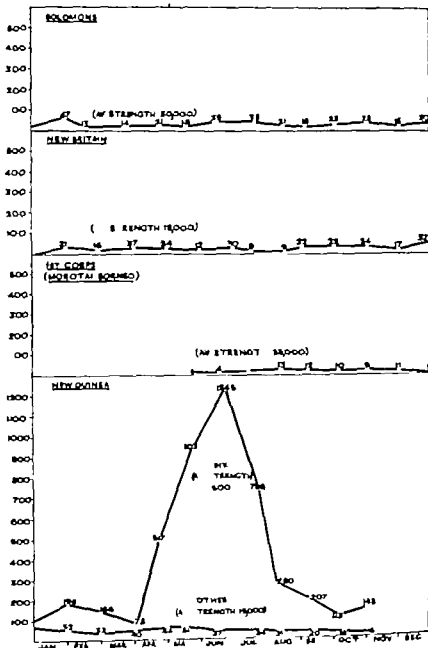


TABLE I  
MALARIA INCIDENCE IN OPERATION AREAS OTHER THAN NEW GUINEA  
(1944-1945)

Location	Period of observation	Total force at risk	Total malaria attacks	Percentage infected
Solomons	27 10 44-5 10 45 (11 months 8 days)	30 000	666	2.2
New Britain	27 10 44-5 10 45 (11 months 8 days)	15 000	277	1.8
Morotai, Borneo	13 4 45-5 10 45 (5 months 22 days)	85 000	113	0.17
Total		130 000	1 256	0.97

highest rate (113.2 per 1,000 per week) developed in the 16th Brigade in May, 1945. By September, 1 month after the end of the war, rates had fallen to a relatively low level. Only four deaths from malaria were recorded during this campaign and none from blackwater fever.

#### (4) THE MALARIA OUTBREAK IN THE AITAIPE-WEWAK AREA

##### (A) MALARIA INCIDENCE

It will be seen from Table II that of a total force of 17,500 men of the 6th Division, engaged in jungle fighting for a period of over 11 months in the Aitaipe-Wewak area, 4,838 individuals suffered from one or more attacks of malaria.

Some 27.6 per cent of the total force developed overt malaria attacks during this period. Judging by past experience it may be assumed that at least 90 per cent of such a force would have acquired M.T. or B.T. malaria or both at some time during this period. If so, then atebirin suppression was effective in suppressing major clinical symptoms in 62.4 per cent over a period of exposure exceeding 11 months.

In three Base Sub-area troops, conditions were more static. Some 539 troops out of 5,500 developed overt attacks, i.e., 9.8 per cent.

##### (B) TYPE OF MALARIA

Details in regard to the type of malaria found are given in Table III. It will be seen that malignant tertian infections were most common, parasites of *P. falciparum* were demonstrated in 78.2 per cent of the total cases (single



TABLE II.  
MALARIA INCIDENCE IN THE AITAPU-WEWAK AREA  
(20th October 1944-29th September 1945)

Formation.	Total force at work.	Total individuals having 1 or more attacks.	Total attacks of malaria.	Average attack per patient.
6th Australian Division	17,800	4,838 (27.2%)	6,285	1.3
3 Base sub-area	5,800	529 (9.1%)	621	1.15
Total	23 600	5,377 (22.8%)	6 906	1.28

and mixed infections). *P. vivax* was found in 12.8 per cent. (single and mixed infections). In 11.1 per cent. the diagnosis was unconfirmed by the finding of parasites. Thus "not confirmed" group included clinical malaria and probably infections like dengue fever and other febrile conditions where the medical officer felt the patient should receive the benefit of the doubt and have anti malaria treatment without further delay.

### (C) MULTIPLE ATTACKS.

It will be seen that the proportion of men suffering from multiple attacks was not high. In troops of the 6th Australian Division the average attack

TABLE III.  
TYPE OF MALARIA FOUND IN 6 906 OVERT ATTACKS IN THE AITAPU-WEWAK AREA  
(20th October 1944-29th September 1945).

Formation	MT	BT	MT and BT	Not con- firmed (clinical diagnosis).	Total attacks.
6th Australian Division	4,779 (78.6%)	886 (14.6%)	133 (2.2%)	703 (11.2%)	6,285
3 Base sub-area	478 (78.9%)	78 (12.5%)	9 (1.4%)	59 (9.5%)	621
Total	5,257 (76.1%)	964 (14.7%)	142 (2.1%)	762 (11.1%)	6 906

per patient was 1.3, and in Base Sub-area troops 1.15 per patient over a period exceeding 11 months (Table II). When it is remembered that this group included recrudescences and relapses as well as new infections the rate was unexpectedly low and quite at variance with the prevalent medical opinion in the Aitape-Wewak area where recrudescences and relapses following treatment were generally regarded as very much more frequent. This point has considerable significance when the prevalence of atebirin-susceptible and relatively resistant strains comes up for consideration.

#### (D) POSSIBILITY OF AN "X" FACTOR

Because the consistent experimental results obtained by L H Q Medical Research Unit at Cairns and the amazingly low rates in Australian troops operating in all other hyperendemic and highly malarious areas had shown the remarkable efficacy of a daily dose of 0.1 gramme of atebirin in suppressing benign tertian malaria and in suppressing and curing malignant tertian malaria, it was generally considered that the atebirin discipline in this Division must have fallen below the standard required by General Routine Orders.

On the other hand, doubts regarding the invariable efficacy of atebirin in a dosage of one tablet (0.1 gramme) daily arose in the 6th Australian Division itself, since certain combatant and medical personnel were convinced that malaria casualties were occurring despite the conviction that atebirin was being taken each day with unvarying regularity.

It was suggested that some "X" factor was responsible for the epidemic. In June, 1945, the Director of Medicine was instructed by the C-in-C, General Sir THOMAS BLAMEY, to proceed to Wewak and investigate the problem. A field section of the L H Q Medical Research Unit was immediately formed and based at Wewak, it included Major I C MACDONALD as O C, Major J I TONGE as Pathologist, and the appropriate technical staff. Co-ordinated investigations were commenced at Wewak by the Field Section and at Cairns by the L H Q Medical Research Unit without delay. Hundreds of specimens of blood plasma for atebirin estimations were flown to L H Q Medical Research Unit, Cairns. Forty carefully selected patients suffering from malaria contracted in the Wewak area were sent to Cairns by ambulance-plane for purposes of special investigation, and in order to infect anopheline mosquitoes and to study local strains for possible atebirin resistance or decreased susceptibility.

Three major factors had to be considered:

1. Was atebirin being supplied in the correct dosage and being taken daily with unvarying regularity as laid down in General Routine Orders?
2. Was atebirin being absorbed normally and was an adequate blood concentration being built up in troops taking atebirin in the Wewak area?
3. Did atebirin-resistant strains of *P. falciparum* or strains showing

decreased susceptibility to atebryn exist in the Aitape-Wewak area, and if so to what extent were they responsible for the epidemic?

These combined investigations at Wewak and on the mainland showed that —

1 The atebryn tablets being issued at Aitape and Wewak contained not less than an average of 0.1 gramme of the drug, that the tablets dissolved satisfactorily and that the absorption and concentration of the drug in the blood of soldiers at Wewak taking 0.1 gramme of atebryn daily was normal, i.e. that it was similar to that occurring in volunteers at Cairns.

2 The consumption of atebryn by certain individual soldiers sent from Wewak to Cairns was not always up to standard for in two patients who had suffered from repeated attacks of malaria, plasma atebryn estimations indicated they were somehow purposely avoiding taking atebryn. When it was suggested that they would be held indefinitely in the North if malaria attacks continued, a dramatic rise in atebryn levels resulted, the concentration being subsequently maintained.

In Wewak, on the other hand, the atebryn-plasma levels in a large series of soldiers taking 0.1 gramme daily were found to be similar to those observed in volunteers at Cairns (21.6 micrograms per litre). The plasma atebryn levels found in the 6th Australian Division during this late period of observation, i.e. July-September indicated that atebryn consumption at that time was in general, adequate to confer protection against all strains of *P. falciparum* previously worked with at Cairns.

3 In twenty apparently healthy men in the Wewak area, malaria parasites were demonstrated in the blood while they were taking the prescribed amount of atebryn (0.1 gramme daily) and showing a mean atebryn concentration of 21.1 micrograms per litre. Such a result had never been observed even in hyperinfected volunteers at Cairns and similar malaria surveys showed it was not happening to troops operating in other hyperendemic areas.

It remained to consider whether atebryn-resistant strains or strains of *P. falciparum* with decreased susceptibilities to atebryn existed in the Aitape-Wewak area. The subsequent communication deals with this aspect of the investigation.

#### (5) ATEBRIN SUSCEPTIBILITY OF AITAPE WEWAK STRAINS OF *P. falciparum*

The strains of *P. falciparum* previously used in the experiments at Cairns were obtained from soldiers evacuated from New Guinea. These soldiers had served in Buna-Gona, Huon Peninsula, Wau or Milne Bay campaigns in

Chemical and pharmaceutical investigations of the atebryn tablets being issued in Aitape and Wewak were undertaken by Dr. ADRIEN ALBERT, University of Sydney.

New Guinea, all these areas were hyperendemic and situated south to south-east of the Aitape-Wewak area

The main investigation was to determine if there existed a strain (or strains) of *P falciparum* with a relative insusceptibility or insensitivity to atebryn in volunteers or soldiers taking the normal suppressive dosage of this drug

Experimental infections specifically designed to investigate strain differences were exclusively mosquito-transmitted

#### (A) MATERIAL INVESTIGATED

Soldiers from the Aitape-Wewak area were specially selected for investigation before being sent to Cairns either by the Director of Medicine or by Medical Officers in the Aitape-Wewak-Lae area acting on his instructions. The basis of selection was —

- (1) The presence of heavy blood infection or hyperinfection
- (2) Some anomaly in the history or clinical picture of the patient
- (3) Frequent attacks of falciparum malaria, reasonably suspected of being recrudescences

Selected patients subsequently were sent to Cairns by air ambulance, having minimal quinine therapy in transit. In this connection it should be emphasized that typical cases, which comprised the majority of hospital admissions for malaria in the Aitape-Wewak area, were not specifically included in this particular investigation, as it was assumed their strain of parasite would be of the usual atebryn-susceptible type. The Aitape-Wewak strains therefore came from a highly selected group of individuals.

In the early stages of the investigation mosquitoes were infected with *P falciparum* obtained from each soldier admitted with overt malaria. Subsequently it proved more economical to feed mosquitoes only on those soldiers who had gametocytes in their peripheral blood rather than specifically to produce gametocyte carriers.

Table IV (p. 240) sets out the essential features of the histories of the soldiers who provided the falciparum gametocytes which were used to infect mosquitoes (Details in Appendix A).

It will be seen that six of the nine soldiers selected had more than one attack of malaria. Furthermore, in these six soldiers there were twenty-two attacks of malaria over an aggregate period of 60 weeks—an average of slightly less than 3 weeks between attacks. As standard therapy\* (Q A P M) was stated to have been given for each attack it is not unreasonable to assume that

\* Standard therapy (Q A P M) at Aitape-Wewak at this time consisted of quinine sulphate as a mixture (30 grains daily) for 3 days, followed by atebryn dihydrochloride 0.6 gramme daily for 2 days, atebryn dihydrochloride 0.3 gramme daily for 3 days, atebryn dihydrochloride 0.2 gramme and plasmoquine base 20 mg daily for 3 days. This was followed by either a maintenance dose of atebryn of 0.1 or 0.2 gramme daily for 42 days if the individual was leaving malarious areas or, if remaining, by the usual suppressive regimen of 0.1 gramme daily indefinitely.

the majority of these attacks were recrudescences. In contrast to this, the average number of malaria attacks per patient in the Aitape-Wewak area was 1.15 to 1.3 over a period exceeding 11 months. This figure included relapses, recrudescences and fresh infections.

In the records of one soldier No. 7 who was admitted with hyperinfection, 20 per cent. of the red blood cells were parasitized clinically his condition was one of early cerebral malaria.

Soldiers Nos. 620 and 2 were selected for Cairns because it was observed that in them *P. falciparum* appeared to be relatively unsusceptible to atebryn.

TABLE IV

GAMETOCTYE CARRIERS USED TO INFECT MOSQUITOES FOR EXPERIMENTAL SPOROBLASTIC TRANSMISSION OF AITAPE-WEWAK STRAINS OF *P. falciparum*.

No.	Name.	Unit.	Malaria attacks before transfer to Cairns.	Service in Wewak area in weeks.		Admitted to Cairns.	Serial suppressive atebryn mg./day
				Total	Before first malarial attack.		
8	LEE.	2/11 Bn.	4 in 18 weeks	33	17	17.45	140
4	GRA.	3/1 Bn.	4 in 16 weeks	24	14	16.645	100
6	DER.	2/9 Cdo.	1 in 1 week	30	29	16.645	140
620	STE.	3/11 Bn.	6 in 14 weeks	28	11	11.645	100
2	CRE.	2/5 Bn.	4 in 9 weeks	4	18	16.645	140
19	TEE.	2/3 M/G Bn.	3 in 9 weeks	28	19	18.745	100
30	ANS.	14 F'd. Bty	2 in 2 weeks	29	26	5.845	160
22	WOO.	2/1 F'd. Regt.	1 in 1 week	30	25	26.745	140
7	PER.	2/10 Cdo.	1 in 1 week	32	31	17.45	100

—suppressive atebryn failing to protect and standard Q.A.P. treatment failing to cure them. They were selected as being most suitable for the isolation of a strain or strains which might prove relatively unsusceptible to chemotherapeutic agents. In addition to these soldiers many others were studied with regard to response to therapy with paludrine and to suppressive atebryn. Some were investigated for atebryn utilization—a progressive decline in atebryn-plasma concentrations combined with other evidence indicated that in two patients atebryn intake was being purposely avoided. The first evidence obtained at Cairns of a relatively atebryn-unsusceptible strain was encountered in one of these men. This individual (No. 2 CRE) had had four attacks in 9 weeks and the question naturally arose whether a relatively atebryn-unsusceptible strain could be developing in him as a result of prolonged suboptimal dosage.

## (B) ISOLATION OF STRAINS

Good gametocyte waves developed in soldiers Nos 7, 8, 19, 22 and 30 and mosquitoes fed on them became infected.

Owing to other investigations which were being carried out at the time, soldiers Nos 620, 24 and 6 were subinoculated rather than allowed to develop their own gametocyte waves. The recipients of their blood developed trophozoite waves and were partially treated, subsequently gametocyte waves developed and mosquitoes feeding on these recipients became infected.

Little difficulty was experienced in infecting mosquitoes from these soldier—gametocyte production did not appear to differ from that observed simultaneously in volunteers infected with "old" New Guinea strains or with strains from New Britain.

## (C) EXPERIMENTS PERFORMED

The first experiments were performed with single strains of *P. falciparum* but subsequently mixed strains were used to make the conditions of the experiments more stringent. Tables recording data concerning the infected batches of mosquitoes and the number of infective bites are recorded in Appendix B.

In all experiments, I to VI, the chemotherapeutic agent was administered as a daily dose at 10.00 hours (10 a.m.). Before exposure to infection a "build-up" was given which varied with the drug being used, and, with the exception of atebryn, was the standard "build-up" always adopted at Cairns. The "build-up" for atebryn was either 200 mg daily for 7 days (100 mg daily maintenance dose) or 400 mg daily for 7 days (200 mg daily maintenance dose). The daily administration of drugs was continued over the biting period and for 28 days after the last infective bite provided the volunteer did not develop overt malaria requiring therapy. Tables recording the plasma-atebryn values in volunteers used in Experiments I to VI will be found in Appendix C.

## (D) RESULTS OF EXPERIMENTS (I TO VI)

For convenience in describing the chemotherapeutic effects of drugs in volunteers exposed to malaria, the degree of protection afforded the volunteer is classified as follows—

*Class I effect* = complete protection. This is either complete causal prophylaxis or complete suppression and radical cure.

*Class II effect* = complete suppression but not radical cure, overt malaria developing after cessation of suppressive regimen.

*Class III effect* = partial suppression, i.e., parasites appear in small numbers in thick films, but overt malaria does not develop until after the cessation of the suppressive regimen.

*Class II effect* = failed suppression, i.e., development of overt malaria within 28 days of exposure to infection and while taking suppressive drug.

*Experiment I (Aisape-Wewak strains)*

Pairs of volunteers, one having atebryn 100 mg daily and the other having no drug therapy, were bitten by mosquitoes at a single session on day 0. The batches of mosquitoes

were infected with sporozoites derived from one gametocyte carrier. One pair of volunteers was bitten by mosquitoes infected with one of the nine strains being investigated, nine pairs were used in all.

Infection.—Single strains of *P. falciparum* ten infective bites on day 0. (Detail in Appendix B.)

Atebrin.—A build-up of 200 mg daily for 7 days, then 100 mg daily to the 24th day after exposure unless therapy was required for an overt attack.

TABLE V

SUPPRESSIVE ALUE OF ATEBRIN (0.1 GRAMME DAILY) IN NINE VOLUNTEERS EACH INFECTED WITH SPOOROZITES DERIVED FROM NINE DIFFERENT CARRIERS HARBOURING AN AFRICAN WILKES STRAIN OF *P. falciparum*.

Strain No.	Volunteer having no drug.			Volunteer having atebria.				Degree of protection (Class I-IV).
	First demonstrable parasitaemia.	Overt malaria.	First therapy.	Mean plasma-atebrin level gammae/litre	First demonstrable parasitaemia.	Overt malaria.	First therapy.	
Days since exposure.				Days since exposure				
5	9	12	18	1	8	14	17	IV
4	10	12	18	16	12	1	20	IV
6	8	11	12	1	1	12	18	IV 200 mg. atebria daily from day 18-47. Class II effect.
620	8	11	12	22	12	—	26	III. 200 mg daily from day 24-25. Class I effect.
2	9	12	15	17	27	—	29	III Q.A.P. M. followed by radical cure.
10	10	1	14	23	—	—	43	II
30	11	12	18	48	—	—	62	II
22	9	12	14	21	—	—	—	I
7	8	10	1	27	—	—	—	I

Atebrin 100 mg daily continued to the 24th day after exposure

#### Comments

Volunteers exposed to strains Nos. 4, 8 and 6 all developed demonstrable parasites and overt malaria whilst taking 100 mg atebria daily (Class IV effect)

The volunteer exposed to strain 2 developed demonstrable parasites on

the last day but one of administration of 100 mg atebirin daily. Overt malaria developed after ceasing suppression (Class III effect).

The volunteer exposed to strain No 620 had demonstrable parasites during the period of administration of 100 mg atebirin daily (56 days), when the dose of atebirin was subsequently increased to 200 mg daily and continued for 28 days complete suppression and radical cure resulted (Class III effect with 100 mg daily, Class I effect with 200 mg daily).

Adequate suppression was obtained in four out of the nine volunteers, and of these four, two developed overt malaria after ceasing atebirin administration.

Only two strains, Nos 7 and 22, behaved normally, *i.e.*, were fully suppressed and radically cured in volunteers having 100 mg daily when the dosage was continued for 28 days after exposure to infection (Class I effect). It is worthy of comment that both of these strains were isolated from soldiers who were having their first attacks of malaria, they conformed in type to all strains of *P. falciparum* previously studied from New Guinea or elsewhere in the South-West Pacific.

The mean plasma-atebirin levels in these volunteers were lower than usually observed—this was related to an unsatisfactory "build-up" which was used, *i.e.*, 200 mg atebirin daily for 7 days. In the original atebirin suppression experiments at Cairns 0.1 gramme daily for 4 to 8 weeks was customary. So prolonged a "build-up" was impracticable owing to the urgency of this investigation.

#### Experiment II (*Aitape-Wewak strains*)

Two groups of four volunteers were exposed to intensive infection with sporozoites derived from each of the strains. One group of four volunteers received 100 mg of atebirin daily, the other 100 mg of paludrine daily. As controls of the strains, one volunteer having 100 mg atebirin and one having 100 mg paludrine were included in the group and were exposed, at the same sessions as the other eight volunteers, to infection with sporozoites of a strain of *P. falciparum* obtained from a soldier evacuated directly from New Britain (Bougainville) who had never been to Wewak (Chart 3, p 244).

Infection—Multiple strains of *P. falciparum*, 41 infective bites in eight sessions over 15 days (Detail in Appendix B).

Atebirin—A "build-up" of 200 mg daily for 7 days, then a daily dose of 100 mg continued over the biting period and for 28 days after the last infective bites (Total of 42 days).

Paludrine—A daily dosage of 100 mg commenced the day before first exposure and continued over the biting period and for 28 days after the last infective bites (Total of 42 days).

#### Comment

(a) Three of the four volunteers receiving 100 mg atebirin daily and exposed to Aitape-Wewak strains of *P. falciparum* developed overt malaria (Class IV effect). The infection in the other volunteer was partially suppressed, later the dosage was increased to 200 mg of atebirin daily, but cure did not result (Class III effect).









(c) Volunteers receiving 25, 50, or 100 mg of paludrine daily were completely protected—25 mg or more daily acted as a complete causal prophylactic (Class I effect). Subinoculation from the volunteer receiving 25 mg daily on 7th day after exposure was negative, showing that erythrocytic parasites were failing to reach the circulation.

TABLE VII

VOLUNTEERS RECEIVING ATEBRIN SUPPRESSION AND EXPOSED TO INTENSIVE INFECTION WITH SPOOROZOITES OF *P. falciparum* ISOLATED FROM SEVEN GAMETOCYTE CARRIERS FROM AITAIPE-WFWAK. THREE VOLUNTEERS RECEIVING PALUDRINE SUPPRESSION WERE INCLUDED FOR COMPARISON.

Name	Suppressive drug	Dose in mg / day	Mean <sub>0</sub> plasma-atebrin levels gamma/litre	On suppressive drug		After ceasing suppression		Degree of protection (Class I-IV)
				First demonstrable parasites	Overt malaria	First demonstrable parasites	Overt malaria	
				Days since first exposure				
HIT	Atebrin	100	22.5	12	19	—	—	IV
ROB	Atebrin	200	23.0	12	—	—	53	III
GIM	Atebrin	200	23.7	36	—	—	47	III
WIR	Atebrin	200	39.8	—	—	56	60	II
O'D	Paludrine	100		—	—	—	—	I
CRA	Paludrine	50		—	—	—	—	I
ILL	Paludrine	25		—	—	—	—	I

#### Experiment IV (Aitaipe-Wewak strains)

A group similar to Experiment III was used except that there were included four instead of three volunteers having atebrin 200 mg daily. The infection, however, was light and was limited to six infective bites over a period of 26 days (Chart 5, p. 248).

Infection—Six infective bites received in three sessions of two bites each in a period of 26 days. All mosquitoes were dissected after engorging (Detail in Appendix B).

Atebrin—100 mg or 200 mg daily continued over the biting period and for the subsequent 28 days, total of 53 days. A "build-up" of 200 mg daily for 7 days was adopted for the 100 mg regimen and 400 mg daily for 7 days for the 200 mg regimen.

Paludrine—The daily dose commenced 1 day prior to exposure and continued over the period of exposure and subsequent 28 days (Total of 53 days).

#### Comment

(a) The volunteer having 100 mg of atebrin daily developed demonstrable parasites, and overt malaria requiring therapy, after ceasing atebrin administration (Class III effect).







Sulphadiazine—A "build-up" of 1,000 mg daily for 4 days, then 500 mg daily, continued over the biting period and subsequent 28 days  
 Sontochin and resochoin—A "build-up" of 400 mg daily for 4 days, then 100 mg administered daily and continued over the period of exposure and for the subsequent 28 days

### Comment

- (a) The volunteer receiving 100 mg of atebtrin daily and the volunteer having quinine sulphate, grains x daily, developed demonstrable parasites and overt malaria requiring therapy (Class IV effect) In a later experiment it was found in two volunteers taking quinine sulphate (grains x daily) and two taking grains xxx daily, that suppression of all major symptoms and final cure of a relatively atebtrin-insusceptible strain had resulted
- (b) In one of the volunteers receiving sontochin (100 mg daily) major symptoms were completely suppressed but demonstrable parasites and overt malaria developed after ceasing sontochin administration (Class II effect)
- (c) In one volunteer receiving sontochin 100 mg daily, in one having sulphadiazine 500 mg daily and in both having resochoin 100 mg daily, malaria was fully suppressed and radically cured (Class I effect)

TABLE IX  
 VOLUNTEERS HAVING ATEBRIN QUININE, SULPHADIAZINE, SANTOCHIN OR RESOCHIN EACH DAY WERE EXPOSED TO MOSQUITOES INFECTED WITH TWO STRAINS OF *P. falciparum* ISOLATED FROM GAMETOCYTE CARRIERS FROM AITAIPE-WEWAK

10 MOSQUITOES INFECTED WITH TWO STRAINS OF <i>P. falciparum</i> ISOLATED FROM GAMETOCYTE CARRIERS FROM AITAIPE-WEWAK								
Name	Suppressive drug	Dose in mg / day	Mean plasma-atebrin levels gamma / litre	On suppressive drug		After ceasing suppression		Degree of protection (Class I-IV)
				First demonstrable parasites	Overt malaria	First demonstrable parasites	Overt malaria	
Days since first exposure								
WAL	Atebrin	100	24.8	18	18	—	—	IV
CHI	Quinine	670		10	19	—	—	IV
MAC	Sulphadiazine	500		—	—	—	—	I
POS SAL	Santochin Santochin	100 100		— —	— —	— —	— —	II I
FRA. HAR	Resochin Resochin	100 100		— —	— —	34 —	34 —	I I



## Experiment VI (Atetris-Herak strains).

A group of six volunteers was exposed to heavy infection with single strain of *P. falciparum* (strain No. 8). One volunteer received 100 mg. atetris daily two 100 mg. sontochin daily two 50 mg. resochin daily and one 500 mg. sulphadiazine daily (Chart 7).

Infection.—Single strain of *P. falciparum* ten infective bites received in one session on day 0.

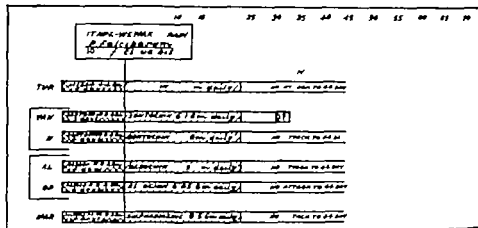
Atetris and sontochin.—A build-up of 400 mg. daily was given for 4 days. A daily dosage of 100 mg. was continued over the day of exposure and for the subsequent 23 days.

Resochin.—A "build-up" of 200 mg. daily was given for 4 days. A daily dosage of 50 mg. was continued over the day of exposure and for the subsequent 23 days.

Sulphadiazine.—500 mg. daily commencing 4 days prior to exposure and continued over the day of exposure and for the subsequent 23 days.

## CHART 7 (Experiment VI).

ACTION OF ATETRIS 0.1 SONTOCHIN 0.1 RESOCHIN 0.05 AND SULPHADIAZINE 0.5 GRAMS DAILY ON VOLUNTEERS EXPOSED TO *P. falciparum*.



## Comment.

(a) The volunteer receiving 100 mg. of atetris daily both volunteer receiving resochin 50 mg. daily and the volunteer receiving 500 mg. of sulphadiazine daily developed no major symptoms of malaria while receiving their suppressive regimens. During a period of 29 days observation after ceasing to take drugs, none of these volunteers developed either clinical or laboratory evidence of malaria.

As no volunteer receiving suppressive drugs and exposed to New Guinea strains of *P. falciparum* has had a period of freedom from malaria for longer

than 19 days unless he was radically cured of his infection, it was considered that these volunteers had been radically cured

(b) One of the two volunteers receiving sontochn 100 mg daily developed overt falciparum malaria 7 days after ceasing his suppressive regimen. In both volunteers major symptoms were completely suppressed and one was radically cured of his infection

TABLE X.

VOLUNTEERS HAVING ATEBRIN, SONTOCHIN, RESOCHIN OR SULPHADIAZINE SUPPRESSION WERE EXPOSED TO MOSQUITOES INFECTED WITH A STRAIN OF *P. falciparum* ISOLATED FROM A GAMETOCYTE CARRIER FROM AITAIPE-WEWAK. (STRAIN NO 8)

Name	Suppressive drug	Dose in mg / day	On suppressive drug		After ceasing suppression		Degree of protection Class
			First demonstrable parasites	Overt malaria	First demonstrable parasites	Overt malaria	
			Days since first exposure				
TUR	Atebrin†	100	—	—			I
VAN	Sontochin	100	—	—	30	31	II
LEN	Sontochin	100	—	—			I
SAL	Resochin	50	—	—			I
COP	Resochin	50	—	—			I
MAR	Sulphadiazine	500	—	—			I

\* Group observed for 29 days after ceasing suppressive regimen

† Mean<sub>G</sub> plasma-atebrin levels gamma/litre = 22.2 gamma per litre

## (E) DISCUSSION

### (1) Strain differences

Throughout the report "strain" refers to *P. falciparum* isolated from single gametocyte carriers and does not necessarily imply immunological differences

In the first experiments, when individual strains were used to infect volunteers having atebrin 100 mg daily, two of the nine strains behaved normally and were found to be susceptible to this dose of atebrin, infection in the two volunteers being fully suppressed and radically cured. The two gametocyte carriers providing these strains were experiencing their first attacks of falciparum malaria. Of the other seven gametocyte carriers providing the relatively insusceptible or resistant strains, six had had multiple attacks of malaria in the Aitape-Wewak area.

It is of interest to arrange the strains in order of their apparent atebri-  
 n susceptibility based on the results of the first experiment—that is on their  
 behaviour when used alone in one volunteer having atebri-100 mg daily. The  
 development of overt malaria whilst taking atebri-100 was considered to indicate  
 relative insusceptibility or resistance (Class IV effect) demonstrable erythro-  
 cytic parasites while having atebri-100 but no overt malaria till after suppression  
 ceased as the next degree of relative insusceptibility or resistance (Class III  
 effect) complete suppression without radical cure as the third degree of relative  
 insusceptibility or resistance (Class II effect) and finally complete suppression  
 and radical cure indicating normal atebri-100 susceptibility (Class I effect). In  
 Table XI the strains are arranged in order of decreasing insusceptibility to  
 atebri-100, together with the experimental groups of volunteers exposed to them,  
 and the results of the experiments summarized.

It will be seen that the strains can be separated into two main groups.

(a) Relative insusceptibility or resistance to atebri-100 as indicated by Class  
 IV, III or II effect in volunteers having 100 mg of atebri-100 daily

(b) "Normal" susceptibility to atebri-100 as indicated by Class I effect  
 in volunteers having 100 mg atebri-100 daily

TABLE XI

RELATIVE INSUSCEPTIBILITY OF NINE STRAINS OF *P. falciparum* TO ATEBRIN ADMINISTERED  
 DAILY TO VOLUNTEERS EXPOSED TO EXPERIMENTAL SPOROZOITE-INDUCED INFECTION.

Rebath number- ing group.	Screen No.	No. of malaria attacks in donor of strain.	Experimental groups exposed to infection	Class effects I-IV	
				Degree of protection in subjects receiving daily doses of atebri-100	
				100 mg daily	200 mg daily
A	8	4	I II III V VI	9/10 Class IV	2/3 Class III
	4	4	I II		1/3 Class II
	6	1	I II III	1/10 Class I	
A	220	3	I II III IV	3/3 Class III	4/4 Class I
	2	4	I II III	2/3 Class II	
	19	3	I II III IV		
	30	2	I II IV V		
B	23	1	I II III	2/2 Class I	
	7	1	I II III		

This strain had been passed four times through mosquitoes before being used to  
 infect volunteers in Group VI when Class I effect (normal susceptibility) was observed.  
 This may have depended on reversion of the original relatively atebri-100-insusceptible strain  
 to one of normal atebri-100 susceptibility

It is apparent that there are two main types of strain—one relatively insusceptible to atebri $\dot{n}$  and the other normally susceptible. The number of volunteers exposed to each strain or to each group of strains is probably too small to assess degrees of variation due to behaviour of individual volunteers. Nevertheless, there is some evidence of variation in the degree of susceptibility to atebri $\dot{n}$  amongst the relatively insusceptible strains. This suggests that there may have been several strains of *P. falciparum* occurring naturally in the Aitape-Wewak area or that the phenomenon of atebri $\dot{n}$  insusceptibility was not so much an inherent characteristic of the strains as one which had been, or was, in the process of being acquired. It should be emphasized that no atebri $\dot{n}$ -fast strains, similar to the arsenic-fast strains produced by suboptimal dosage of various arsenicals in trypanosomiasis, have been encountered. As already noted, possible evidence of loss of atebri $\dot{n}$  insusceptibility was obtained in experiments with a strain which has involved four passages through mosquitoes.

Four volunteers were treated with standard Q A P M course of therapy, three of these had been exposed to Group A strains (Table X) and were in Experiment II—all recrudesced whilst having atebri $\dot{n}$  maintenance (100 mg daily). One volunteer from Experiment I who was infected with Strain 2 in Group A (Table X) was treated with this course of Q A P M and was radically cured.

For comparison a strain of *P. falciparum* isolated from a soldier evacuated from New Britain was transmitted by infected mosquitoes to a volunteer having atebri $\dot{n}$  100 mg daily. This strain was found to behave exactly as did the "old" strains of *P. falciparum* used in earlier experiments at Cairns, *i.e.*, complete suppression and radical cure was obtained in volunteers having 100 mg atebri $\dot{n}$  daily provided the dose was continued for 28 days after last exposure to infection (Class I effect.)

## (2) Plasma-Atebri $\dot{n}$ Concentrations and the Daily Dose of Atebri $\dot{n}$ (100 to 200 mg)

All estimations were made on plasma using the double extraction method of BRODIE and UNDERFRIEND. As remarked earlier in the report, the "build-up" used for the early groups of volunteers was unsatisfactory—the mean<sub>o</sub> plasma-atebri $\dot{n}$  levels at the first exposure to infection were significantly below the mean equilibrium levels observed later. However, this observation was relatively unimportant as later groups had adequate plasma-atebri $\dot{n}$  levels but behaved in precisely the same manner with regard to degree of suppression, further, the infections in some volunteers receiving atebri $\dot{n}$  (200 mg daily) were inadequately suppressed.

It was found that the higher the mean<sub>o</sub> plasma atebri $\dot{n}$  concentration the greater the degree of suppression. The table below sets out the time of development of demonstrable parasites and overt malaria in relation to mean<sub>o</sub> plasma-atebri $\dot{n}$  levels. The volunteers included in this table are all those who were

exposed to infection with strains in Group A, Table VI producing Class IV effect in volunteers having 100 mg of atebirin daily in fact, all volunteers exposed to intensive infection plus the one volunteer exposed to light infection in Experiment V.

It is apparent that the higher the mean<sub>0</sub> plasma atebirin level the longer the period of adequate suppression and the more delayed is the onset of overt malaria. There was one exception to this generalization, namely in the last volunteer receiving 100 mg of atebirin daily whose mean plasma-atebirin level was only 23.0 microgrammes per litre, for Class I effect, i.e. suppression

TABLE VII.

RELATION BETWEEN THE MEAN<sub>0</sub> PLASMA-ATEBIRIN LEVEL AND DEGREE OF SUPPRESSION OBTAINED IN VOLUNTEERS EXPOSED TO *P. falciparum* SPOROZOITES.  
(METHOD: BROOKS & UNDERWOOD)

Mean <sub>0</sub> plasma- atebirin level gmms/ litre	Daily dose of atebirin.  Mg	Days since first exposure to last day of atebirin administration.	Days since first exposure to development of first		Degree of protection.  Class.
			Demon- strable parasites.	Overt malaria.	
9.8	100	19	12	15	IV
18.9	100	17	12	18	IV
20.6	100	28	16	22	IV
22.6	100	20	12	19	IV
23.0	200	42	12	23	III
24.8	100	19	14	18	IV
29.9	100	42	19	23	IV
33.7	200	42	30	47	III
36.8	200	42	54	60	II
22.4	100	23	—	—	I

and radical cure resulted. He had been exposed to Strain 8 (*P. falciparum*) after it had been through four passages in mosquitoes, and, as already indicated, a possible explanation was that the strain had reverted to one of normal atebirin susceptibility.

With regard to the daily dosage of atebirin those volunteers having 200 mg of atebirin daily were better suppressed than those having 100 mg daily. Parasites were demonstrated in two of the three during atebirin administration but overt malaria did not occur in any of these volunteers until after the cessation of suppressive atebirin (Class III and II effects). As had been mentioned in former reports, it is considered that, in general, there is better suppression with 200 mg atebirin daily than with 100 mg daily even though the mean<sub>0</sub>

plasma-atebrin level may be higher in a particular volunteer having 100 mg daily than in one having 200 mg daily

It is clear that 100 or 200 mg of atebriin daily was inadequate for the full suppression and radical cure of all Group A strains of *P. falciparum* with the exception of one volunteer (Experiment VI) infected with Strain 8 after four passages. It is of interest to note (Table IV) that Strain 620 was only partially suppressed by 100 mg daily (Class III effect), but when the dose was increased to 200 mg daily for 28 days there was full suppression and radical cure (Class I effect)

### (3) Intensity of Infection

Experiment IV was used to determine if differences could be observed in the degree of protection afforded to volunteers by 100 or 200 mg of atebriin daily, when the dose of sporozoites was diminished

Table XIII briefly sets out the differences observed between the volunteers exposed to intensive and to light infection

TABLE XIII

DEGREES OF SUPPRESSION OBSERVED IN VOLUNTEERS HAVING 100 OR 200 MG OF ATEBRIN DAILY AND EXPOSED TO EITHER INTENSIVE OR LIGHT INFECTION

Atebrin mg/day	Number of infective bites	Group number	Number of volun- teers exposed	Degree of protection			
				Class			
				IV	III	II	I
100	42 in 8 sessions over 15 days	II III	5	4	1	0	0
100	10 in 1 session on 1 day	VII	1	0	0	0	1*
100	6 in 1 session on 1 day	V	1	1	0	0	0
100	6 in 3 sessions over 26 days	IV	1	0	1	0	0
200	42 in 8 sessions over 15 days	III	3	0	2	1	0
200	6 in 3 sessions over 26 days	IV	4	0	0	0	4

\* This volunteer was exposed to Strain 8, which had four passages before being used to infect him. Class I effect was possibly due to loss of atebriin insusceptibility

In Table XIII it appears that, in volunteers receiving a given daily dose of atebriin, diminishing the intensity of the infection results in more satisfactory suppression of malaria and a greater likelihood of producing radical cure. However, reference to Table X shows that the volunteers in Experiment IV were not exposed to all the strains most likely to be least susceptible to atebriin—this factor appears to be more important than the lightness of the infection.

The number of volunteers was small but those exposed to light infection

had higher mean<sub>0</sub> plasma atebirin levels than those exposed to intensive infection, as is shown in Table XIV.

It cannot be said there was definite evidence that light infection was the factor responsible for the relatively good results.

TABLE XIV  
MEAN<sub>0</sub> PLASMA-ATEBRIN LEVELS IN VOLUNTEERS EXPOSED TO LIGHT OR INTENSIVE INFECTION WITH MULTIPLE STRAINS OF *P. falciparum*.

Intensive infection.			Light infection.					
Mean <sub>0</sub> plasma-atebrin level grains/litre	Atebrin dosage (mg./day).	Degree of protection.  (Class)	Mean <sub>0</sub> plasma-atebrin level grains/litre	Atebrin dosage (mg./day).	Degree of protection.  (Class).			
8.6	100	IV	20.3	100	III			
18.9	100	IV						
20.6	100	IV						
22.5	100	IV						
23.6	200	III	27.9	100	IV			
23.9	100	IV						
31.7	200	III				34.0	200	I
						41.0	200	I
33.8	200	II	48.3	200	I			

#### (4) Susceptibility to Chemotherapeutic Agents other than Atebrin.

All volunteers having paludrine, whether in dosage of 25, 50 or 100 mg daily were fully protected against *falciparum* malaria—the action of paludrine being that of a complete causal prophylactic. (Class I effect.)

Quinine sulphate (grains x) administered to one volunteer failed to prevent the appearance of *P. falciparum* parasites and the development of overt malaria. A similar failure of quinine (grains x) to suppress *P. falciparum* infections has been noted with all other strains tested. (Class IV effect.)

Sixteen volunteers receiving sontochin 100 mg daily had been exposed to *P. falciparum* (intensive infection) in earlier experiments using atebirin-susceptible strains and all had been completely suppressed and radically cured. (Class I effect.) The failure to produce radical cure in two of four volunteers used in the present experiment may be taken as presumptive evidence of diminished susceptibility of the Aisaape-Wewak strains to sontochin.

Eight men taking resochin, (chloroquin) 100 mg daily had previously been exposed to intensive *P. falciparum* infection using atebirin-susceptible strains

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all the infections were completely suppressed and radically cured. Similar results were obtained with two out of two volunteers receiving 100 mg and two out of two receiving 50 mg of resochin daily when infected with Aitape-Wewak relatively atebtrin-insusceptible strains. As the dosage equivalent of resochin is one-half that of atebtrin, for purposes of comparison it was necessary to compare the results obtained with 200 mg of atebtrin daily. None of the strains showed any increased resochin resistance.

No evidence of decreased susceptibility to sulphadiazine was obtained in the two volunteers in these groups receiving 500 mg daily, the infection was both suppressed and radically cured. All previous volunteers in our series tested against sulphadiazine with atebtrin-susceptible strains had received 1.0 gramme daily with similar results.

With the possible exception of sontochin no evidence of decreased susceptibility or increased resistance on the part of the Aitape-Wewak strains to anti-malaria drugs other than atebtrin has been found. It is a matter of chemotherapeutic interest that a strain of parasite showing relative resistance to atebtrin and sontochin should manifest no increased resistance to resochin when used in equivalent dosage. Had it been feasible further observations would have been made to confirm these findings as the number of volunteers used was unavoidably small, but the time factor, i.e., the end of the war—precluded this being done.

#### 6 ATEBRIN SUSCEPTIBILITY IN AN AITAPE-WEWAK STRAIN OF *P. vivax*

A group of six volunteers was used—four received atebtrin 700 mg weekly and two received 400 mg weekly. Atebtrin (100 mg) was administered daily to the volunteers receiving 700 mg and to the volunteers receiving 400 mg weekly each Monday, Tuesday, Thursday and Saturday. These dosages were followed over the period of exposure and for the subsequent 23 days. The "build-up" for the 700 mg weekly group was 400 mg daily for 4 days followed by 100 mg daily for 11 days prior to exposure. The "build-up" for the 400 mg weekly group was 1,400 mg given over a period of 5 days followed by 400 mg weekly for 7 days prior to exposure.

Infection. Thirty *A. punctulatus punctulatus* infected with viable sporozoites of *P. vivax* (Aitape-Wewak strain) were allowed to engorge on each volunteer. There were four biting sessions over 10 days, five infective bites were given on day 0, ten on day +3, five on day +6, and ten on day +9. The batches of mosquitoes were 50 to 72 per cent infected, the salivary gland infections light to medium-heavy, and the sporozoite age from 2 to 6 days. The strain used in these experiments was derived from SIM, who was a gunner in an artillery battery. The fact that there was an interval of 14 months without an attack of malaria between the two campaigns is accepted as indicating the attack on 14.10.45 was the result of a fresh infection (*P. vivax*) acquired in the Wewak area. History is detailed below.



*Clinical history*

Sgt. Gunner in artillery battery

*Service in Aitape Newak area* Seven months (2nd January 1945 to 25th August, 1945).

*Stated Suppression.* Atebrin 100 mg. daily

*Previous malaria attacks* (1) *Previous campaigns* eight attacks (2 M.T. and six B.T. (between February 1943 and April, 1944). (2) *Present campaign*, two attacks (i) *P. vivax* 14th June 1945 (ii) *P. vivax* 22nd August, 1945 (present attack).

*Present attack.* Stated to be having 200 mg. of atebrin daily since last attack, 14th June, 1945. The first symptoms occurred on 22nd August, 1945 and he developed a moderate attack of malaria. Parasites (*P. vivax*) were demonstrated in his blood films on 23rd August, 1945 the plasma-atebrin was 19 micrograms per litre. This level was undoubtedly due to recent dosage with atebrin. At Cairns, when he arrived it was noted that there was only a trace of atebrin staining of his skin the spleen was enlarged two to three finger breadths below the left costal margin and the haemoglobin value was 13.4 grammes per 100 c.c. A good gametocyte wave subsequently developed and some mosquitoes were fed, but difficulty was encountered because of the patient's lack of co-operation and unwillingness to delay therapy for his attack. A full course of paludrine treatment was given.

## ANALYSIS OF RESULTS OF THE EXPERIMENT

1. *During period of Atebrin administration.*

None of the six volunteers developed either clinical malaria or demonstrable parasites during the period of atebrin administration. No differences were observed between the four volunteers receiving 700 mg. atebrin weekly and those receiving 400 mg. atebrin weekly. Minor clinical features were conspicuous by their absence. (Detailed laboratory examinations were not carried out on these volunteers.)

Mean plasma atebrin concentrations were estimated and found to be —

(i) 700 mg. atebrin group 17.0 to 22.9  $\mu$ g. per litre and a group mean of 20.9  $\mu$ g. per litre.

(ii) 400 mg. atebrin group 14.0  $\mu$ g. per litre

2. *After ceasing Atebrin administration*

All six volunteers developed overt malaria and demonstrable parasites (*P. vivax*) after ceasing to take atebrin. The first parasites were demonstrated in the volunteers who received 700 mg. of atebrin weekly between 25 and 33 days after the last dose, while overt malaria appeared 28 to 35 days after the last dose. The two volunteers who had received 400 mg. weekly developed demonstrable parasites and overt malaria 17 and 23 days after the last dose of atebrin respectively.

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TABLE XV

ESSENTIAL FEATURES OF A GROUP OF VOLUNTEERS RECEIVING EITHER 400 MG OR 700 MG OF ATEBRIN WEEKLY AND EXPOSED TO EXPERIMENTAL MOSQUITO-TRANSMITTED *P vivax* (AITAIPE-WEWAK STRAIN)

Name	Atebrin suppression		During suppression		Days after ceasing suppression to			Plasma atebryn	
	Dose mg / week	Duration (days)	Demonstrable parasites	Minor clinical features	Demonstrable parasites	Temperature to 100° F	Overt malaria	During suppression	Appearance first parasites
O'S HUG HOO SLA	700	— 11 to + 32	—	—	27	26	28	21 1	11
	700	— 11 to + 32	—	—	29	28	30	17 0	4
	700	— 11 to + 32	—	—	25	28	28	22 9	4
	700	— 11 to + 32	—	—	33	35	35	23 3	7 5
JO JL	400	— 9 to + 31	—	—	17	16	17	14 0	6
	400	— 9 to + 31	—	—	23	20	23	14.0	0

The plasma-atebrin concentration on the day of first demonstrable parasites varied from 0 to 11  $\mu\text{g}$  per litre with a mean<sub>o</sub> of 4.5  $\mu\text{g}$  per litre

#### Comment

The suppression of the major symptoms of B T malaria has been shown both experimentally and by experience to require less atebryn than does M T malaria

None of the six volunteers infected with this Aitaape-Wewak strain, whether receiving four or seven tablets of atebryn weekly, developed symptoms until 17 to 33 days after ceasing taking the drug. Suppression was complete and demonstrable parasites were absent from the blood throughout the period of drug administration. Similar results have been recorded with all strains of *P vivax* so far investigated. No evidence of a relatively atebryn-insusceptible strain of *P vivax* has been obtained either at Wewak or elsewhere.

In operational areas it was anticipated that *vivax* rates would be low, occurring only in that small proportion of troops who for various reasons fail to take even four tablets weekly.

A comparison is made of the incidence of confirmed *P vivax* malaria in the different campaigns of 1944-45 in the accompanying Table XVI.

In addition, in the Wewak area there were 144 instances of mixed B T and M T infections, compared with 14 in the Solomons, 1 in New Britain and 1 in Morotai and Borneo.

It will be noted that overt attacks of *P vivax* malaria were much more frequent in the Aitaape-Wewak area than the Solomons or New Britain. Troops

in Morotai were at slight risk, while those in Borneo were probably mainly exposed to risk while in the jungle—further the period of exposure in both places was less—just under 6 months. Even so as it was known that many of these troops had been previously infected with *P. vivax* in former campaigns their low rate was an outstanding achievement as was the rate in the forces in the Solomons and New Britain, where the malaria hazard was great. The fact that the *P. vivax* rate in troops in the Aitape-Wewak area was so much greater than corresponding rates in the Solomons and New Britain, which were comparable hyperendemic areas of malaria, indicates that their standard of atebirin discipline was inferior.

TABLE XVI.

THE INCIDENCE OF CONFIRMED B.T. MALARIA IN DIFFERENT CAMPAIGNS (1944-1945).

Location.	Total admissions ( <i>P. vivax</i> )	Total force at risk.	Admission rate per 1,000.
Aitape-Wewak	541	22,000	24.2
Solomons	278	30,000	9.3
New Britain	290	18,000	12.3
Morotai and Borneo	180	22,000	3.1

## 7 SUMMARY AND CONCLUSIONS.

1 Volunteers receiving various chemotherapeutic agents have been experimentally infected with mosquito-transmitted strains of *P. falciparum* obtained from nine soldiers with natural infection acquired in Aitape Wewak, New Guinea.

2 The strains were derived from a highly selected group of patients specially chosen for investigation at Cairns because of the tendency to recrudescence after standard Q.A.P. M treatment, or for some other reason such as hyperinfection. They were exceptional cases and did not represent a typical cross section of malaria as it manifested itself in the Aitape Wewak area.

3 The strain or strains of *P. falciparum* obtained from seven of these nine soldiers were atypical inasmuch as malaria was not suppressed in volunteers experimentally infected with these strains when receiving 100 mg. of atebirin daily and was sometimes suppressed but not readily cured in volunteers similarly infected when receiving 200 mg. of atebirin daily despite the fact that both of these suppressive regimens were continued for 28 days after the last exposure to infection.

4 The strain of *P. falciparum* obtained from two of the nine soldiers was completely suppressed and radically cured in volunteers receiving 100 mg. of atebirin daily over the period of exposure and subsequent 28 days. Both of these soldiers had had only one attack of malaria.

5 Four volunteers developing overt malaria at Cairns were treated with the standard course of therapy employed in the Aitape-Wewak area. Three of these four volunteers were not radically cured by this course of therapy even though it was followed by maintenance atebirin—0.1 gramme daily. Such a result was most unusual and had been only very rarely observed previously. The significance of this and similar observations in regard to the prevalence of this strain throughout the Aitape-Wewak campaign was considerable. Since the incidence per man of secondary attacks was only 1.15 to 1.3 throughout the Aitape-Wewak campaign, it follows that failure to establish radical cure with standard Q A P M treatment was exceptional and that the common strain producing malaria there was the atebirin-susceptible and not the relatively atebirin-resistant strain.

6 Evidence was obtained that the greater the daily dose of atebirin or the higher the mean plasma-atebirin level, the greater the degree of protection afforded to volunteers exposed to infection with the relatively atebirin-resistant strain or strains of *P. falciparum* found at Wewak.

7 There appeared to be two main groups of "strains" of *P. falciparum* with regard to their susceptibility to atebirin in volunteers receiving 100 or 200 mg daily.

*Group A Strains*—Three strains regularly produced overt falciparum malaria in volunteers receiving 100 mg of atebirin daily, and were sometimes suppressed, but not radically cured in volunteers receiving 200 mg of atebirin daily. Four strains were partially or completely suppressed, but not radically cured by 100 mg of atebirin administered each day, but were completely suppressed and radically cured in volunteers receiving 200 mg of atebirin daily. These relatively atebirin-insusceptible or atebirin-resistant strains have been encountered nowhere else in New Guinea or New Britain and only accounted for a minority of the malaria casualties in the Aitape-Wewak area.

*Group B Strains*—Two strains were completely suppressed and radically cured in volunteers receiving 100 mg of atebirin daily. This atebirin-susceptible strain was the common strain affecting troops in the Wewak area and conformed in behaviour to all other strains of *P. falciparum* encountered in New Guinea or New Britain.

8 Paludrine in dosage of 25 to 100 mg daily acted as a complete causal prophylactic against all these nine strains of *P. falciparum*. It is evident that had paludrine been available at the time for field use, malaria casualties due to relatively atebirin-resistant or insusceptible strains would have ceased.

9 With the exception of atebirin and sontochin, no evidence of decreased susceptibility or increased resistance on the part of Aitape-Wewak strains to anti-malaria drugs (quinine, resochin and sulphadiazine) was found.

10 The mode of origin of the Aitape-Wewak relatively atebirin-resistant or atebirin-insusceptible strain is a matter of conjecture and has not been finally determined. The quality of relative atebirin-resistance may have arisen suddenly as a mutation in soldiers taking atebirin, or constituted an inherent biological characteristic of a geographically limited strain. On the other hand, the fact that it was first demonstrated in an individual who was purposely avoiding taking atebirin, suggested it might be originating as a result of prolonged suboptimal dosage. Suggestive evidence of loss of atebirin resistance

was obtained in Experiment VI with the relatively insusceptible or resistant Strain 8 after four passages. After the fourth passage through mosquitoes in a volunteer exposed to ten infective bites (*P. falciparum*) suppression and cure resulted with a mean plasma-atebrin level of only 23.0 microgrammes per litre while on a regimen of 0.1 gramme of atebrin daily.

11 Volunteers infected with an Aitape Wewak strain of *P. vivax* failed to develop major symptoms of malaria or parasites in the blood while taking four or seven tablets of atebrin weekly. No evidence of an atebrin-insusceptible or atebrin-resistant strain of *P. vivax* was obtained. The 741 attacks of overt benign tertian malaria developing in troops in the Aitape Wewak area must be attributed to their failure to take even four tablets a week.

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#### APPENDIX A.

##### HISTORIES OF SOLDIERS USED AS GAMETOCYTE CARRIERS FOR INVESTIGATION OF WEWAK STRAINS OF *P. falciparum*

No. 8 LEE. Pte. in infantry battalion. Aet. 20 years.

Served in Aitape Wewak area from November 1944, to July 1945—8 months.

Stated suppression: Atebrin 100 mg. daily.

Previous malaria attacks: (1) 30.3.45—*P. falciparum*—Q.A.P. M  
 (2) 5.5.45—*P. falciparum*—Q.A.P. M  
 (3) 31.5.45—Unconfirmed—Q.A.P. M  
 (4) 24.6.45—*P. falciparum*—quinine

*Present attack* 24.6.45. One trophozoite *P. falciparum* per field in thick blood film. Stated to have ended course of therapy for last attack 8 days ago, also stated that maintenance atebrin commenced 1 day ago (*i.e.* 7 days without maintenance atebrin). On 6th quinine sulphate in tablet to Camra. Ceased quinine on 30.7.45 after receiving total dose of 150 grams in 8 days. On 7.7.45 (1 week after admission) *P. falciparum* trophozoites of the order 1 per  $\mu$ m were demonstrated. Densities increased to 22,000 per c.mm. and moderate attack of malaria occurred. A gametocyte was reaching maximum density of 160 per c.mm. was observed and *A. punctulatus punctulatus* were readily infected.

The soldier was treated with atebrin while the gametocytes were present. He received 1.6 grammes over period of 16 days. Paludrine 300 mg. daily was then given for 10 days with rapid response and cure of the infection.

Twenty-three days after the last dose of paludrine trophozoites of *P. vivax* were demonstrated in thick blood films—overt vivax malaria requiring therapy developed.

## No 4 GRA Pte in infantry battalion Aet 24 years

Served in Aitape-Wewak area from December, 1944, to June, 1945—6 months

Stated suppression Atebrin 100 mg daily

Previous malaria attacks (1) 4 4 45—*P falciparum*—Q.A.P M  
(2) 5 5 45—*P falciparum*—Q.A.P M  
(3) 21 5 45—*P falciparum*—Q.A.P M  
(4) 2 6 45—*P falciparum*—Q.A.P M  
(5) 16 6 45—*P falciparum*—

*Present attack* 16 6 45 Began to feel "off colour" towards the end of his course, and on arrival at Cairns had 240 trophozoites of *P falciparum* per c mm (in thick blood films) He was given quinine sulphate 20 grains on 19 6 45, and 10 grains on 20 6 45, which controlled the attack His general condition was not good and treatment with paludrine 100 mg daily for 7 days was commenced on 29 6 45 with rapid and satisfactory response Parasite densities reached a maximum of 3,980 per c mm on 28 6 45, at which time the soldier had a moderate attack of *P falciparum* malaria On the day of first therapy (29 6 45) 16 per c mm trophozoites of *P vivax* were seen as well as 1,780 per c mm *P falciparum* This soldier did not develop a satisfactory gametocyte wave No *P falciparum* gametocytes were seen and only four *P vivax* gametocytes on 27 45

A secondary attack of vivax malaria occurred on 22 7 45, 17 days after the last dose of paludrine 100 mg daily

Subinoculation of 10 c c whole blood was made from this soldier into a non-immune volunteer for the purpose of obtaining a gametocyte wave adequate for the infection of *A punctulatus punctulatus*

## No 6 DER L/cpl in Commando squadron Aet 29 years

Served in Aitape-Wewak area from November, 1944, to June, 1945—7½ months

Stated suppression Atebrin 100 mg daily

Previous malaria attacks (1) 13 6 45—*P falciparum*—quinine

*Present attack* 10 6 45 Admitted to hospital in Aitape-Wewak with the diagnosis of "irritable colon in a neuroasthenic unstable personality" Complaints—typical of neuroasthenic individual, who is anxious Developed falciparum malaria while in hospital, on the 4th day, and was given quinine 10 grains daily for 2 days before arrival at Cairns On arrival (16 6 45) there were 800 per c mm *P falciparum* trophozoites and 16 gametocytes per c mm He was given quinine sulphate 90 grains over a period of 14 days and the infection was controlled though the soldier remained partly confined to bed He was subinoculated on 26 6 45, when parasite densities in the peripheral blood were 4,500 per c mm The recipient of his blood (2 c c) developed a good gametocyte wave and infected mosquitoes satisfactorily

Paludrine 100 mg daily was administered from 7 7 45 to 13 7 45—a total of 7 days, with a satisfactory clinical and parasitological response, 18 days after the last dose, overt malaria developed and both *P vivax* and *P falciparum* trophozoites were demonstrated in thick films during the ensuing attack

## No 620 STE Pte in infantry battalion Aet 39 years

Served in Aitape-Wewak area from November, 1944, to May, 1945—6 months

Stated suppression Atebrin 100 mg daily

Previous malaria attacks (1) 21 1 45—*P falciparum*—Q.A.P M  
(2) 11 2 45—*P falciparum*—Q.A.P M  
(3) 28 2 45—*P falciparum*—Q.A.P M  
(4) 13 3 45—*P falciparum*—Q.A.P M  
(5) 6 4 45—*P falciparum*—Q.A.P M  
(6) 30 4 45—*P falciparum*—Q.A.P M  
(7) 15 5 45—*P falciparum*

It is noted in this soldier's records for the fifth and sixth attacks that atebrin was being taken and that the urinary atebrin concentration exceeded 10 micrograms per litre

22. IF00 Dvr in transport platoon. *Act* 29 years.

Served in Aitape-Wewak area from October, 1944 to July 1945—9 months.

Stated suppression 100 mg. atebirin daily but till 1 month ago 200 mg. daily.

Previous attacks of malaria: (1) 19th July 1945—*P. falciparum*—quinine.

*Present attack* First attack of malaria, moderate severity. Given quinine 10 grains 19th July and 22nd July which controlled the infection. *P. falciparum* trophozoites reached density of 12,000 per c.mm. on 28th July and gametocytes reached maximum of 4,000 per c.mm. on 31st July when paludrine was commenced in dosage of 300 mg. daily for 10 days.

Plasma-atebrin level 20th July = 18 µg per litre.

No. 7 PER. Tpr in Commando squadron. *Act* 23 years.

Served in Aitape-Wewak area from November 1944 to July 1945—8 months.

Stated suppression 100 mg atebirin daily

Previous attacks of malaria: (1) 22.7.45—*P. falciparum*—quinine

*Present attack*: Hyperinfection—stated to have 20 per cent. 1 red cell infected. Given intravenous quinine and intramuscular atebirin 22nd, 23rd and 24th July 1945, followed by quinine sulphate 40 grains daily by mouth for 4 days—followed by atebirin 200 mg. for 1 day then quinine 10 grains for 1 day. On 8th day after arrival at Casua (10.7.45) *P. falciparum* and *P. vivax* trophozoites were demonstrated in thick blood films. On 13.7.45 *P. falciparum* trophozoites numbered 41,000 per c.mm. and atebirin 300 mg was given daily for 3 days. A good gametocyte was developed, maximum count of 1,000 per c.mm. being reached on 8th August. Batches of mosquitoes were fed on this soldier and were satisfactorily infected. Treatment was with paludrine 300 mg. daily for 10 days, concluding on 3.8.45. A secondary attack of vivax malaria occurred on 27.8.45.

## APPENDIX B.

DETAIL OF INFECTIVE BITES GIVEN TO VOLUNTEERS IN EXPERIMENTAL GROUPS TO 1.  
(AITAPE-WEWAK STRADA)

## EXPERIMENT 1.

Strain number	Passages	Number of infective bites.	Batch numbers.	Infective rates of bunches.	Gland infections.	Sporozoite age in days.
				Per cent.		
8	8	10	A 21	76	Heavy	8
4	T8	10-11	991	100	Medium	10
6	T8	9-10	A 18	77	Medium	8
820	T8	10	1000	80	Heavy	11
2	T8	11-12	993	100	Heavy	10
			994	100	Heavy	10
19	8	10	A-23	81	Heavy	12
30	8	10	A 53	83	Heavy	4
23	8	10	A-34	83	Heavy	12
7	8	10-11	A-20	84	Heavy	12

Average = 9-12 infective bites

T = trophozoite transmission    S = sporozoite transmission

## EXPERIMENT II

Day of exposure to infection	Strain number	Passages	Number of infective bites	Batch numbers	Infective rates of batches	Gland infections	Sporozoite age in days
					Per cent		
0	1	TS	3	A-12	85	Medium	15-18
				A-1	75	Light	15-18
0	2	TS	2	A-2	68	Heavy	18
+ 2	7	S	10-11	A-10	85	Medium	15-17
				A-20	94	Heavy	
				A-16	95	Heavy	
+ 4	8	S	4	A-21	80	Heavy	12
+ 6	19	S	3-4	A-23	88	Heavy	16
				A-13	80	Medium	10
+ 8	22	S	4-5	A-25	96	Heavy	16
				A-27			
+ 10	30	S	5	A-52	85	Heavy	4
				A-15			
+ 12	620	TS	4-5	A-50	80	Heavy	6
+ 14	4	TS	5-6	A-46	93	Medium	7

Average = 41-43 infective bites

## EXPERIMENT-III

Day of exposure to infection	Strain number	Passages	Number of infective bites	Batch numbers	Infection rates of batches	Gland infections	Sporozoite age in days
					Per cent		
0	620	TSS	5	A-102	75	Heavy	4
				A-103	70	Heavy	2
+ 2	22	SS	5	A-99	90	Heavy	6
+ 4	6	TSS	5-6	A-105	87	Heavy	5
				A-106	80	Heavy	2
+ 6	2	TSS	5	A-112	60	Medium	4
+ 8	19	SS	5-6	A-127	77	Medium-heavy	3
+ 10	7	SS	5-6	A-128	75	Medium	3
+ 12	620	TSS	5-6	A-105	100	Heavy	12
+ 14	8	SS	5-6	A-136	93	Heavy	3

Average = 41-42 infective bites

\* T = trophozoite transmission S = sporozoite transmission



## EXPERIMENT IV

(All mosquitoes engorging were dissected.)

Day of exposure to infection.	Strain number	Passages.	Number of infective bites	Batch numbers.	Infection rates of batches.	Gland infections.	Sporozoite age in days
0	250	T88		A-89	85	Heavy	4
+ 20	19	SS		A 133	83	Heavy	9
+ 25	30	SS	1	A 161	90	Heavy	4
	(2) †	(SS) †	1	A-136	100	Heavy	4
8 infective bites (dissected)							

T = trophozoite transmission. S = sporozoite transmission.

## EXPERIMENT V

Day of exposure to infection.	Strain number	Passages.	Number of infective bites.	Batch numbers	Infection rates of batches.	Gland infections.	Sporozoite age in days
0	8	SS	1-2	A 136	85	Heavy	45
	30	SS	2-4	A-161	7	Heavy	13
	(2) †	(SS) †		A 165	100	Heavy	13

Average = 6-7 infective bites

S = sporozoite transmission.

† This strain was obtained from a volunteer who had been exposed to several old strains and Strain 2 from Aburipe-Wewak. It is possible that Strain 2 is responsible for the gametocyte wave which caused the infection in batch A 164 used in this experiment.

## EXPERIMENT VI.

Day of exposure to infection.	Strain number	Passages.	Number of infective bites.	Batch number	Infection rates of batch.	Gland infection.	Sporozoite age in days.
0	8	SSSS	13	A-311	8	Medium	4

S = sporozoite transmission.

## APPENDIX C

PLASMA-ATEBRIN LEVELS—EXPERIMENTAL GROUPS I TO VI  
(AITAIPE-WEWAK STRAINS)

## EXPERIMENT I

(Build-up 200 mg atebnin daily for 7 days)

Strain number *	Volunteer	Atebrin mg / day	Mean <sub>G</sub> plasma-atebrin level gamma/litre	Number of readings	Duration of atebnin administration in days	Plasma-atebrin level at first demonstrable parasites
8 S	JAC	100	20.7	7	36	19
4 TS	BAT	100	18.0	4	29	11
6 TS	LON	100	20.6	7	29	24
620 TS	ROB	100	21.2	25	68	21
2 TS	PYL	100	17.1	12	38	21
19.S	MEN	100	20.6	11	50	11
30 S	HOW	100	46.8	13	44	30
22 S	HAL	100	20.9	13	56	
7 S	STE	100	18.4	12	35	
9 volunteers		100 mg	22.1/μg /litre	104		

\* T = trophozoite transmission S = sporozoite transmission

## EXPERIMENT II

(Build-up for 100 mg atebnin daily = 200 mg daily for 7 days)

Strain type	Volunteer	Atebrin mg / day	Mean <sub>G</sub> plasma-atebrin level gamma/litre	Number of readings	Duration of atebnin administration in days	Plasma-atebrin level at first demonstrable parasites
Aitaape-Wewak	WIN	100	9.6	8	25	11
	PEN	100	18.6	8	23	19
	CRO	100	20.6	12	34	23
	SUM	100	28.9	17	48	28
Bougainville	GAR	100	17.9	18	48	
5 volunteers		100 mg	19.5/μg /litre	63		

## Experiment III

(Build-up for 100 mg. atebrein daily = 200 mg. daily for 7 days).

(Build-up for 200 mg. atebrein daily = 400 mg. daily for 7 days).

Volunteer	Atebrein mg./day	Mean <sub>0</sub> plasma-atebrein level gamma/litre.	Number of readings.	Duration of atebrein administration in days.	Plasma-atebrein level at first parasites in blood films.
HIT	100	22.6	7	22	4
ROB.	200	22.0	10	40	10
GIL	200	22.7	10	40	
WIL	200	22.8	10	40	

## Experiment IV

(Build-up for 100 mg. atebrein daily = 200 mg. daily for 7 days).

(Build-up for 200 mg. atebrein daily = 400 mg. daily for 7 days).

Volunteer	Atebrein mg./day	Mean <sub>0</sub> plasma-atebrein level gamma/litre	Number of readings.	Duration of atebrein administration in days.	Plasma-atebrein level at first parasites in blood films.
MIL	100	20.2	24	0	17
STO.	200	28.2	4	0	
GOB.	200	24.0	24	0	
TAY	200	41.0	24	0	
THO	200	44.2	4	0	

## Experiment V

(Build-up for 100 mg. atebrein daily = 400 mg. daily for 4 days)

Volunteer	Atebrein mg./day	Mean <sub>0</sub> plasma-atebrein level gamma/litre	Number of readings	Duration of atebrein administration in days.	Plasma-atebrein level at first parasites in blood films
WAL	100	27.9	1	4	23

## EXPERIMENT VI

(Build-up for 100 mg atebryn daily = 400 mg daily for 4 days)

Volunteer	Atebryn mg / day	Mean <sub>G</sub> plasma atebryn level gamma/litre	Number of readings	Duration of atebryn administration in days	Plasma- atebryn level at first parasites in blood films
TUR	100	22.4	11	23	—

MEAN<sub>G</sub> PLASMA-ATEBRIN LEVELS(i) *Suppressive regimen of 100 mg daily*

Eighteen volunteers, 221 observations    Observations made over periods of time up to 68 days after cessation of "build-up"

Mean<sub>G</sub> plasma-atebryn level = 21.4 gamma/litreRange of mean<sub>G</sub> plasma-atebryn levels = 9.6 to 46.8 gamma/litreMean<sub>G</sub> of mean<sub>G</sub> plasma-atebryn levels = 20.7 gamma/litre(ii) *Suppressive regimen of 200 mg daily*

Seven volunteers, 153 observations    Observations made over periods of time up to 60 days after cessation of "build-up"

Mean<sub>G</sub> plasma-atebryn level = 35.4 gamma/litreRange of mean<sub>G</sub> plasma-atebryn levels = 23.0 to 46.3 gamma/litreMean<sub>G</sub> of mean<sub>G</sub> plasma-atebryn levels = 34.8 gamma/litre



## CONTROL OF PLAGUE BY MEANS OF LIVE AVIRULENT PLAGUE VACCINE IN SOUTHERN AFRICA (1941-44)

by

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In a previous publication (GRASSET, 1942) an account was given of the first series of field immunizations with live avirulent plague vaccine in the control of plague in South Africa.

During the years 1941-44 there were periodic plague epizootics among wild and domestic rodents throughout the enzootic area. The enzootic area extends widely, from the Cape Karoo and the High Veld, north-westward through the Kalahari desert to Bechuanaland, South-West Africa and Angola.

These epizootics resulted in recurrent plague outbreaks, mainly in the rural Native population, in the northern and southern Free State and Basutoland, the Native territory in the Glen Grey and St. Mark districts, on the border of the Transkei and the Utensberg district. Finally, in South-West Africa, a sharp plague recrudescence occurred, in 1943, in the northern territory of Ovamboland, where plague has been endemic during the past 12 years †.

Following the satisfactory results from live avirulent plague vaccine in 1940-1941 during plague outbreaks, the Union Public Health Department decided to apply this prophylactic method wherever the epidemiological situation appeared to call for active immunization measures.

From January, 1942, to September, 1944, live plague vaccine was used in the control of fourteen plague outbreaks, ten in South Africa, three in Basutoland.

\* We wish to express our thanks to Dr. PETER ALLAN, Secretary for Public Health, for facilities granted in this work and for the data kindly supplied by medical officers of his department.

We are indebted to Brigadier A. ORENSTEIN, Director-General of Medical Services, U.D.F., for his permission to publish data referring to the South African Forces, and to Dr. E. H. CLUVER, Director of the South African Institute for Medical Research, for his personal interest.

We also wish to record our grateful thanks to Dr. HAMILTON DAVEY, Director of Medical Services, Basutoland, for his valuable information, the District Surgeons and Medical Officers of Health who helped us, Mr. D. H. S. DAVIS, Ecologist and Chief Rodent Officer, Public Health Department, Dr. D. F. MURRAY, Plague Diagnostic Department of this Institute for his helpful co-operation, and Miss M. ROBINSON, Serum Department, for technical assistance.

† For data on the history of plague in South Africa reference may be made to MITCHELL, PIRIE and INGRAM (1927) and the *Annual Reports of the Union Public Health Department* (1941-43). For South-West Africa, see the *Report presented by the Government of the Union of South Africa to the Council of the League of Nations concerning the Administration of South-West Africa for the Year 1932*.

and one in South West Africa. Of these outbreaks nine were bubonic and septicaemic, three pneumonic and two had mixed bubonic, pneumonic and septicaemic forms of infection. This does not take into account sporadic epizootic foci at the occurrence of which prophylactic immunization was applied on a limited scale.

As a result of the close co-operation with the Medical Officers of the Union Public Health Department and the Rodent Control Department, steps were taken, wherever possible, to obtain all useful data regarding these various outbreaks. All suspected plague specimens were sent to the plague department of this Institute for bacteriological identification and pathological sections. These included rodents found dead, or caught alive in the suspected areas also postmortem material from human cases of plague or those suspected of having succumbed as a result of that infection.

Bacteriological isolation of *Pasteurella pestis* was carried out wherever possible and followed by virulence tests on susceptible laboratory animals, mainly multimammate mice (*Mastomys caucha*). This South African rodent has been found to be highly susceptible to plague infection (five to six virulent plague bacilli are sufficient to provoke by intraperitoneal injection the death of 90 per cent. of the animals so injected. (DAVIS and MURRAY 1945). When *M. caucha* was not available guinea-pigs were used for virulence tests.

As soon as an outbreak was notified a supply of live plague vaccine was sent by the most rapid route. In many cases vaccine was taken personally from this Institute by public health officers in charge of control of the outbreak. This close co-ordination between the various departments concerned, resulted in the most beneficial results from the epidemiological, hygienic, and immunological viewpoints. When this personal contact could not be arranged, as in the case of an outbreak in remote Native territory the tentative diagnosis of infection was based on epidemiological and clinical evidence and direct smears from bubo or sputum. Supplies of plague vaccine were sent pending bacteriological confirmation. District surgeons and public health officers concerned were sent formularies referring to the main epidemiological and clinical data of the outbreaks, for return to this Institute on termination of the outbreak.

*Origin, i.e.* rodent or human. Local or imported case. Date of commencement of outbreak. Specimens sent for bacteriological examination.

*Number of Plague Cases and Deaths*—Bubonic, pneumonic, septicaemic, with epidemiological and clinical data among non immunized persons.

*Date of Immunization*—Number of persons vaccinated and nature of vaccinal reactions observed.

*Number of Plague Cases and Deaths*—Bubonic, pneumonic, septicaemic among immunized persons.

A. With epidemiological and clinical individual data, *i.e.* respective dates and period between immunization and infectious contact.

B. Period between infectious contact, onset of disease, and death.

*Therapeutic Treatment*—Antiplague serum—sulphonamides

*Additional Data*—Comparison in the evolution of infection between immunized and non-immunized persons, etc

#### TYPE, CONCENTRATION AND DOSES OF VACCINE USED

The vaccine consists of a live emulsion of avirulent strains of *Salmonella pestis* in a concentration of 1,000 million organisms per c c. This is obtained from the 24-hour growth on nutritive agar at 37° C, suspended in saline, from the two following selected strains of avirulent *S. pestis* and adjusted in equal bacterial concentration—

1 Strain K/120 South African avirulent strain of *Salmonella pestis* studied by PIRIE and GRASSET (1941) and found experimentally to possess high immunological properties for guineapigs and rats. Immunological experiments carried out by the writer with MURRAY and DAVIS have shown that it also confers a high active plague protective immunity on multimammate mice (*Mastomys caucha*) against subsequent infection by virulent *S. pestis*.

2 E V avirulent *S. pestis* strain, selected by GIRARD (1935) in Madagascar and used by him in the preparation of avirulent vaccine at Tannanarive, Pasteur Institute. The latter strain has been found by us to be of somewhat lower protecting value in the immunization of rats and multimammate mice against virulent South African strains of *S. pestis* which we have tested. It was, however, kept as a precautionary measure in the preparation of the vaccine, as the latter was used in the immunization of troops in the Madagascar Expeditionary Force.

Immunization consisted of a single injection of 1 c c of avirulent vaccine, administered subcutaneously. This standard dose was given to all adults and old people. Proportionally lower doses were used for children: 0.5 c c in children from 5 to 12 years and 0.25 c c in children under 5 years. The vaccine was used during the month following its preparation. Repeated experiments have shown that when kept in a refrigerator at +2 + 4° C, the biological properties of the live vaccinal emulsion show but little alteration during this period of storage.

#### KEEPING PROPERTIES OF DEHYDRATED VACCINAL EMULSION *in vacuo*

Concentrated live vaccinal emulsions were also dried *in vacuo* at low temperature by means of the lyophile process by Dr LEWIS, of this Institute, and were tested after varying periods of storage in refrigerator—respectively after 3 months, 6 months, 1 year and 2 years. The dry bacterial material was made into an emulsion with saline solution. Part of the respective emulsions were planted on nutritive agar, and the remainders were used for immunization of multimammate mice (*Mastomys caucha*), being given as one single injection of 400 millions subcutaneously. Some delay in the original growth was observed, especially on plain agar. Sub-cultures, however, grew in the usual time and



showed the usual microscopic characteristics. Immunization of multumammu mice with respective samples and with freshly prepared emulsions of stock avirulent vaccine, showed only little attenuation in the antigenic properties of the "dried" vaccine, even with samples used after 2 years of dry storage.

The same process is used for storing individual avirulent plague strains. Besides the above named strains two other avirulent strains, i.e. 1/1904 (Pitts and GRANET 1941) and Tjrwidey R (OTTEN 1938) are kept as a standby and their immunological properties are checked periodically.

#### VACCINAL REACTIONS

Of the total of over 40 000 individual doses of avirulent plague vaccine issued since 1941 no untoward reaction or complication attributable to immunization has been recorded. Clinical information and reports to hand, concerning over 14 000 immunized persons, all emphasize the mild type of vaccinal reactions observed. No racial difference has been noted among Europeans, Natives and Eurasians, nor any difference between sexes.

*Local reactions* Erythema with slight tenderness of about 1 to 3 cm. diameter at the site of injection was commonly observed, usually disappearing within 48 hours. Reaction of the axillary gland was usually absent. Slight and transitory tumefaction of axillary gland was observed in exceptional cases. In some unimmunized persons a hard infiltration at the site of the injection was observed, lasting for a few days.

*General reactions* When present these were limited to a feeling of lassitude or slight headache on the evening of injection without rise in temperature or only a slight rise. Other transitory symptoms observed were pains in the back or more generalized muscular pains, as in influenza lasting for a few hours. These symptoms were accentuated in allergic persons.

The immunization of some 120 public health officers of various municipalities, and the staff of the Rodent Department, carried out by ourselves and by some of the officers from whom we received personal information, proved particularly useful. The majority of these persons were inoculated or re-inoculated on the occasion of a plague outbreak and had to continue with their field work without rest. The inoculation was reported to have resulted in no incapacitation. Clinical records made by some of these medical officers of persons inoculated at the same time as themselves, corroborate their personal observations. The following data referring to various sections of the inoculated population will serve to exemplify these facts.

Dr HAMILTON DYKE, Director of Medical Services, Basutoland, in an interim report dated 4.3.43 on the use of plague vaccine in this territory states:

"The reactions from vaccination were mild, only discomfort and erythema at the site of injection. Two medical officers and I, as well as five European sisters, were immunized, and none of us were incapacitated in our work." Later information from Dr DYKE referring to immunization and re-immunization

of Basutoland Public Health staff, as well as of over 1,100 Natives and 800 men of the African Auxiliary Pioneer Corps, supported this conclusion, *viz.*, reactions among the European staff and Native population immunized were negligible.

#### REACTION OF MINE NATIVES IMMUNIZED

The following clinical observations were made by Dr SPIRO, District Surgeon, Roodepoort (Rand town 21 miles from Johannesburg) from 987 mine Native workers of the South Roodepoort Main Reef Gold Mine, inoculated on 5.9.43, with avirulent plague vaccine.

1 *Temperature* In 886 men temperature was found normal. In 101 slight increase in temperature was observed (one case over  $100^{\circ}\text{F}$ ) as shown in the following temperature record taken 30 hours after inoculation.

Temperature recorded	Number of Native workers immunized
Normal	886
$99^{\circ}\text{--}99.6^{\circ}\text{F}$	69
$99.6^{\circ}\text{--}100^{\circ}\text{F}$	10
$100^{\circ}\text{--}101^{\circ}\text{F}$	0
$101^{\circ}\text{--}102^{\circ}\text{F}$	1

#### 2 *Pain at site of inoculation*

- a Slight or moderate, nineteen, of whom eleven had temperature from  $99^{\circ}$  to  $100^{\circ}\text{F}$ .
- b Severe pain twenty-five, of whom eight had temperature up to  $99^{\circ}\text{F}$ .

#### 3 *Headache*, two temperature $99^{\circ}\text{F}$

#### 4 *General body pain*, two, of whom one had temperature $99^{\circ}\text{F}$

#### 5 *Other signs or symptoms*, nil

#### VACCINAL REACTIONS AMONG INOCULATED TROOPS

The following records, released with the permission of the DIRECTOR GENERAL OF MEDICAL SERVICES, U D F, were taken from 1,212 of 3,600 men inoculated with 1 c.c. of avirulent plague vaccine.

These included 401 Europeans, 615 Natives and 196 Eurasians. There were no untoward reactions and on the whole vaccinal reactions, when present, were very mild. Fifty-one, or 4.2 per cent, had local reactions—swollen and painful arms; thirty-five, or 3.7 per cent, had a general reaction—headache, backache or shivering, generalized body pains, and three with painful axillary gland. Details of the respective occurrence of reactions are given in the following table —

Race and number inoculated.		Local reaction	General reaction.
European	401	18 (3.7 per cent)	19 (4.7 per cent)
Native	813	37 (4.3)	21 (2.2)
Eurafricans	196	9 (4.3)	6 (2)

The low frequency rate of reactions observed among the various sections of the inoculated population (civilian, mining and army) corroborate the harmlessness and safety in large-scale field application of the method, resulting in its easy acceptance by the uneducated Native population.

#### LIVE PLAGUE VACCINE IN THE CONTROL OF PNEUMONIC AND BUBONIC PLAGUE OUTBREAKS

A short account of the plague outbreaks in which live avirulent plague vaccine was used, will now be given, with the main epidemiological and immunological data and the influence of prophylactic vaccination in the evolution of these outbreaks. The fourteen outbreaks will be grouped according to the main clinical types of the infection, *viz.*, bubonic (and septicæmic) and pneumonic, so as to facilitate the respective analyses, especially as regards the influence of immunization in the respective epidemiological forms of the infection.

##### VILJOENSKROON PNEUMONIC PLAGUE OUTBREAK (JULY-AUGUST 1941).

This was the second plague outbreak in this northern Orange Free State district in 1941. The first was a mixed bubonic-pneumonic outbreak in a farming area. This was the first occasion in which live avirulent plague vaccine was used and resulted in rapid control of the outbreak. (GRASSY 1942.)

Four months later (12th July), in the Native location of this small town, where no active immunization had been carried out, pneumonic plague broke out. The original case an old woman who died within 48 hours, was followed by ten further plague cases all pneumonic, occurring within the subsequent 3 weeks *viz.*, six members of the same family and four friends. Eight of these persons lived in the location close to the hut of the original case and two on a neighbouring farm. All the cases were direct, close contacts who visited or nursed the first case, slept in the same hut or attended her funeral. The first case died within 48 hours after the onset of the disease. Sputum of these cases sent to this Institute was found to contain virulent *S. pestis*.

Immunization with live plague vaccine was started on 25th July and continued during the following days. Vaccine was given by Dr CLARK (700 Natives (adults and children) of the crowded now quarantined, population of the location. In addition to the vaccine, 50 c.c. of anti plague concentrated serum was administered to all direct contacts of cases. Children received 25 c.c. These included all persons who had been living in the same hut as

the patient before her death. In one case, two children had slept under the blanket of the infected mother, one right up to the time of her death. Those who, although not in contact with the patient before her death, but who assisted in the burial, were given 25 c.c. anti-plague serum. The total number of immunized, close contacts was estimated at about thirty-five. Of these three developed pneumonic plague. One, a man of about 30 years, fell ill 24 hours after vaccination and died on the 2nd day of illness. Besides 50 c.c. of anti-plague serum injected prophylactically, he was given 300 c.c. of serum therapeutically. In Dr CLARK's opinion (1943) vaccine was injected too late in the incubation period and could hardly be expected to give any protection.

The second case was a man of 35, whose wife had died on 26th July without having received any vaccine. This man had received 1 c.c. of vaccine and serum on the 24th and 25th July. His incubation period was unusually long—12 days, he fell ill on the 7th August and died on the 9th August in spite of further serum treatment. Sputum was *S. pestis* positive.

The third case was that of an old woman of about 70, who also contracted pneumonic plague and *recovered*. Considering the particular interest of this case it will be referred to in more detail. The following data regarding this case have been taken from Dr CLARK's paper (1943). She was a close contact of several fatal cases and had received 1 c.c. plague vaccine and 50 c.c. anti-plague serum prophylactically only 5 days before the acute onset of her illness. Directly she became ill, she was treated with large doses of plague serum. A total of 450 c.c. was given intramuscularly and subcutaneously. She had the same symptoms as the other cases—high temperature with a feeling of constriction amounting to actual pain in the chest, accompanied by a frequent "loose" cough producing a frothy sputum which later contained bright-red blood.

However, after a week of acute illness, she finally recovered. Bacteriological evidence of pneumonic plague was found in the sputum sent to this Institute. Cultural examinations were at first negative. Two guinea-pigs were, however, scarified with the sputum. One developed a bubo and was killed 14 days later. Cultures made from this gland proved to be positive for *S. pestis*. From this culture two further guinea-pigs were scarified and one was inoculated. One of the scarified guinea-pigs died 7 days later, and a section showed plague lesions. *S. pestis* was recovered from the lungs, glands and spleen, and also from the bone marrow. The other guinea-pigs also died. Cultures obtained from these were inoculated into two multimammate mice which died 4 days later. Postmortem sections confirmed death from plague and *S. pestis* was again recovered from the animals. It was therefore proved beyond any doubt that the sputum of this patient contained plague bacilli.

According to Dr CLARK's statement, it is reasonable to suppose that the prophylactic use of the live vaccine and serum, given 5 days before the onset of her illness, and the therapeutic serum treatment caused her recovery. In our opinion we are inclined to credit serum treatment *per se* with little therapeutic

influence in this case, judging from the negative curative results observed in a number of pneumonic cases submitted to large doses of serum prophylactically and therapeutically in the absence of vaccination or jointly with live vaccine but given less than 5 days before the onset of pneumonic plague symptoms. According to Dr LAI TEH WU (1928), anti-plague serum given prophylactically is liable, as in the second immunized fatal case given above, to prolong the incubation period of pneumonic plague. Once the onset of pulmonary symptoms is observed, plague serum is considered by Dr Wu to have very limited therapeutic action, even on the duration of the illness.

On the other hand, our epidemiological experience with avirulent plague vaccine in South Africa in a number of bubonic, septicaemic and pneumonic plague outbreaks has revealed the following facts. The specific protection derived from live vaccine starts to be effective from the 5th day after inoculation. Immunized persons infected during this critical phase and in whom the onset of symptoms appears within the 4 days after inoculation do not benefit from any apparent protection from the vaccine. Those for whom the onset of the infection occurred on the 5th or 6th days after inoculation, show in many cases a less fulminating or a more attenuated form of the disease. This is precisely the case of the old woman who recovered after receiving 1 c.c. of live vaccine and 50 c.c. of serum 5 days before the onset of the illness. Subsequent injections of large therapeutic doses of concentrated plague serum may have contributed to delay the evolution of the infection pending the development of active protection derived from the vaccine, eventually resulting in the jugulation of the infection.

This case marked the end of this pneumonic outbreak. The Native location remained in quarantine until 12 days after the onset of her illness, during which time the old patient was kept under strict guard while the usual disinfection and anti-rodent decontamination measures were applied.

From the epidemiological point of view it is interesting to observe, as pointed out by Dr CLARK, that the majority of people who contracted the disease were old people (eight of twelve). The two youngest were 30 and 40 years. No case was observed among immunized children, several of whom were, however very close contacts. For instance, three, aged 10, 5 and 2 years, slept in the same hut and the youngest slept under the blanket of her mother up to the very day of the latter's death, as she was only seen by a medical officer for the first time that evening. Let us add that this case constitutes the second case of recovery from pneumonic plague after live avirulent plague vaccination. The first case was observed by GALE (1941) and will be referred to later in some detail in the discussion.

#### ANTI PLAGUE IMMUNIZATION IN BASUTOLAND (1942-44).

The following epidemiological data concerning plague immunization in the territory were obtained through the kind personal interest of Dr HAMILTON DYKE, Director of Medical Services in Basutoland.

From March, 1942, to August, 1943 there were four successive plague

outbreaks, regarding three of which we were able to obtain data, two being bubonic one mixed bubonic-pneumonic and one pneumonic. In these outbreaks, live avirulent plague vaccine was used.

*Bubonic outbreak, March 1942* From 18th to 30th of this month, eight cases of typical bubonic plague were observed in three distinct villages about 12 miles distant from Maseru the capital of this territory. All Natives affected showed glandular swelling tense and painful, six with inguinal glands, one cervical and one axillary. Bacteriological examination of fluid from non-suppurating bubo revealed *S. pestis*. Immunization with live avirulent plague vaccine was started on 21st March and included seven medical personnel and fifty immediate contacts. During the three following days the entire populations of the three infected villages were immunized a total of 380 Natives. Vaccinal reactions among adults as well as children were negligible.

Of the six cases which occurred prior to immunization three died within three days of the onset of the disease. Of the 380 persons immunized, two contracted plague. One a child vaccinated on 21st March, fell ill 2 days after inoculation and died 3 days later, i.e. 5th day after immunization. The second case was a girl of 11 years, from a hut where two cases of plague had occurred. She became ill on 29th March i.e. 6 days after immunization. She had no glandular swellings but was very seriously ill, having symptoms of septicaemic plague. She was treated with sulphapyridine (M & B 693) given in dosages of 1 gramme at 6-hourly intervals for 3 days, and recovered.

In Dr DYKE'S opinion, these two cases were probably infected prior to vaccination or on the date of immunization. From the immunological point of view, the first case could not be expected to derive any protection from vaccination as death occurred on the 5th day after inoculation. In the second severe septicaemic case recovery may be attributed to the combined benefit derived from active immunization associated with sulphapyridine treatment.

*The second outbreak (mixed bubonic, septicaemic and pneumonic)* This outbreak commenced on about 2nd January, 1943, and was reported to Dr DYKE on 9th January for, associated with the illness among humans, dead rodents were discovered in the huts of infected cases.

Seven plague cases occurred, all in a village 5 miles from Maseru. There were three deaths, two septicaemic cases before the outbreak was notified and investigated, and one pneumonic case who died in Maseru prior to diagnosis of the disease. Of the remaining cases who recovered, two were bubonic and two septicaemic. One of the latter and one bubonic case were treated with anti-plague serum and sulphapyridine (treatment in the other two cases was not specified). None of these cases had been immunized.

Vaccination with avirulent plague vaccine was carried out on 12th January on eighty persons. Two of the contacts developed acute adenitis 8 days after immunization. They were suggestive of plague but bacteriological examination of fluid from the gland punctures was negative. Both cases recovered. No

further cases of plague were recorded. Plague immunization was also extended to 509 Natives belonging to a unit of the African Auxiliary Pioneer Corps undergoing training in a camp 3 miles from the first affected village. This was considered a necessary precautionary measure, as many of these men, during their off duty time, visited the surrounding villages. In this second series, as in the previous one, vaccinal reactions were very mild. No case of plague occurred among the troops.

*The third outbreak June to July 1943* This was of the pneumonic type and occurred some 50 miles south of Maseru. From the 19th to 23rd June, four deaths occurred in Luchaba's village. Symptoms suggested pneumonic plague. Quarantine was at once established. Specimens of sputum obtained from a further patient on 30th June was sent to this Institute and found positive for *S. pestis*.

On 30th June, four more deaths from pneumonic plague had occurred. On the same day 150 Natives were inoculated with live vaccine. Three days later on 3/7/43 a contact from Luchaba's village, who had been inoculated on 30.6.43 fled to Mapeachaba's village fell ill on the 4th day and died the following day 5/7/43. The village was quarantined and 300 persons were vaccinated on 7th July. No further cases occurred in the latter village. In the case just mentioned, the man could not have benefited from inoculation at that early stage as shown by the date of onset of his symptoms, viz., 4 days after immunization followed the next day by his death.

Between the 30th June the day of vaccination, and the 12th July four more pulmonary cases occurred in Luchaba's village three of whom died and one recovered. All of them had been inoculated on 30th June. The one who recovered fell ill on the 6th day after immunization and recovered in spite of the fact that he did not get anti plague serum or sulphonamide. It is regretted that owing to distances and difficulties in transport, no bacteriological examination of the sputum was possible, although in Dr DYKE's opinion there was full epidemiological and clinical evidence of the plague nature of infection. Owing to the same difficulties no accurate data could be obtained regarding the period between immunization, onset of the disease and death in the three fatal cases. Since 10th July no further plague cases have occurred among contacts in either village.

Summarizing the plague position in Basutoland in a report sent to this Institute on 28.8.43 Dr DYKE concluded "This is the fourth outbreak of plague in Basutoland in which live plague vaccine has been used, one in 1941 and three in 1943. In all of them the outbreak terminated completely within 10 days of inoculation. It is also worth noting that two cases recovered, one septicaemic and one pneumonic they both fell ill on the 6th day after inoculation."

#### IMMUNIZATION DURING PLAGUE OUTBREAKS IN SOUTHERN AFRICA (1942-44)

A short account will now be given of plague outbreaks, eight of which

occurred from 1942 to 1944 in the Union of South Africa and in which anti-plague inoculation with live vaccine was carried out

1 *Stoffberg outbreak (Heilbron district, Orange Free State)*

District Surgeon Dr P VIVIER's report After two cases of bubonic plague in the beginning of March, 1942, 400 persons in this farming district were immunized, including seven contacts Only very slight local reaction, redness and pain at the site of injection, was observed No further case of plague was reported following vaccination

2 *Rouxville outbreak (Orange Free State)*

District Surgeon Dr W JORDAAN's report In March, 1942, following two bacteriologically confirmed cases of plague, one bubonic and one pneumonic, eighty-eight persons, Europeans and Natives, were immunized with live vaccine No further case was observed after inoculation The two original cases were treated with anti-plague serum The bubonic case recovered but the pneumonic case died

3 *Deben outbreak (Kuruman district, Cape Province)*

District Surgeon Dr G E VAN DE MERWE's report, October, 1942 Immunization of 50 persons on farms was carried out after four cases of pneumonic plague had occurred, all of which were fatal No cases were observed among immunized persons Vaccinal reactions were limited to lassitude on the day of injection

4 *Odendaalsrust bubonic plague outbreak (Hoopstad district, Orange Free State)*

Within the days following the 13th January, 1943, three cases of bubonic plague were reported on the farm Middeldam Of these two were fatal Five hundred persons were then vaccinated with live plague vaccine One bubonic case was observed after immunization with the onset of symptoms occurring 3 days (21.1.43) after vaccination, followed by recovery

5 *Steynsrust bubonic plague outbreak (Orange Free State)*

District Surgeon Dr LIEBENBERG's report Plague broke out between the 8th and 13th January, 1943, about 8 miles from Steynsrust Two cases of bubonic plague were identified One case died, showing an unusual bubo localization over the angle of the jaw, the other case recovered Neither of these persons had received vaccine Fifty-seven persons, adults and children, were immunized with live plague vaccine No further case of plague was reported following immunization

6 *Viljoenskroon bubonic plague outbreak (Orange Free State)*

District Surgeon Dr GOLDBERG's report The following bubonic outbreak occurred in a part of the district where two pneumonic outbreaks took place in



1941 as referred to earlier on in this paper. On 11.2.43 Dr GOLDBERG was called to see the first case of bubonic plague, a Native boy aged 7 years. The child had a bubo in the groin although he started complaining of the swelling and the pain only the day before examination, he was already in a very critical condition—opisthotonus and delirium were present. The child was immediately given 50 c.c. of anti-plague serum intramuscularly but died 4 hours later.

On the receipt of live plague vaccine the following day 1.2.43 Dr. GOLDBERG inoculated all contacts on the farm numbering eighteen Natives and four Europeans. A further bubonic case was observed on the 16.2.43, also a child from the hut adjoining that where the first case occurred. The girl, aged 8 had been vaccinated 12.2.43 i.e. 4 days before. A left femoral bubo was present at an early stage. Temperature was 99.2° F., and little pain was experienced by the patient. She was given 50 c.c. anti-plague serum intramuscularly. On 18.2.43 the child's temperature was normal and the bubo was considerably smaller and less painful. Patient was found running about.

In Dr GOLDBERG'S opinion (he has had much experience in plague work) live avirulent immunization accounted for the mildness and speedy recovery of that case. This contrasts with the rapid and severe form of infection in the first unvaccinated child who died 24 hours after commencement of the bubo. Both children were of the same age and infection most likely arose from the same source in both cases. Another interesting point is also to be noted. The absence of further cases after vaccination in spite of the fact mentioned in the Rodent Inspector's report, that the store house, dwelling house and Native huts which were devermured, were infested with plague-carrying fleas.

#### 7. *Cofimaba bubonic plague outbreak (St Mark's district Cape Province).*

During the last days of January 1944 and the first half of February six plague cases, all bubonic, occurred in this Native district. Following this, 300 persons adults and children, in the area where plague occurred, were immunized with live plague vaccine on 19.2.43. Only mild reactions were observed in these persons, including babies under 2 years who received 0.5 c.c. vaccine. No further cases from this district were reported after inoculation during the following 9 months.

#### 8. *Glen Grey bubonic plague outbreak (Lady Frere district Natal)*

From 28th December 1943 and during the month of January 1944 nine cases of bubonic and septicaemic plague were observed in the Lantu Native location of Glen Grey district with four deaths respectively on 31.1.43, 17.1.44, 21.1.44 and 23.1.44. Anti-plague immunization was started on 4.2.44 beginning with twenty contacts. One of the latter developed a bubo during the following week. In spite of immediate serum treatment, the patient died on 13.2.44 i.e., 9 days after immunization. Subsequently 325 Natives

in the location were immunized, after which no further plague case developed in the location

#### ROODEPOORT PLAGUE FATALITY AND PROPHYLACTIC PLAGUE IMMUNIZATION ON THE RAND

Since the severe plague outbreak in Johannesburg in 1904 during which a total of 113 cases with eighty-two deaths occurred, only one isolated sporadic bubonic case of plague (in 1927) has been observed on the Rand during the last 40 years. The very effective anti-plague organization of the Union Public Health Department associated with that of the various Rand municipalities must be credited for the control of plague in this large mining and industrial agglomeration, the total population is 1,000,000 persons, of whom about half are Europeans, the other half being made up of Natives, Eurafrians and Asiatics.

Permanently enforced measures include rodent destruction (trapping, gassing) by plague gangs in the various municipalities under a Central Rodent Inspector. Daily examination of rats and veld rodents caught or found dead in the various areas of the Rand, is carried out, the specimens are sent to the Plague Department of the South African Institute for Medical Research for the detection of eventual plague carriers.

From the middle of July, 1943, manifestations of a plague epizootic appeared evident on the Rand. After isolation of *S. pestis* from three gerbils originating from Zuurbekom, other plague-infected gerbils and rats were caught in various parts of the Rand, at Alberton, Germiston, Paulstruisfontein and the City Deep mine. Some plague-infected rats were actually caught in several parts of the Johannesburg municipal area. From 18 7 43 to 29 43 plague carrier rodents (gerbils and rats) were obtained from twelve different places on the Rand—over a distance of 40 miles.

After consultation with the Public Health authorities of nine Rand municipalities, it was decided that all medical staff, senior and junior, as well as rodent inspectors, European and Native staffs of rodent gangs and all persons connected with anti-plague control, should be submitted to anti-plague immunization with live avirulent vaccine. A total of 120 Europeans, plus Natives of various public health organizations, were inoculated in August and September, 1943.

On 29th August, 1943, a postmortem was performed by Dr. Spiro, Roodepoort District Surgeon, on a Native who had died, at the age of 55 years, 8 miles south of Roodepoort and 21 miles from Johannesburg. This Native complained of feeling out of sorts 3 days prior to his death. He complained of a headache, tightness in the chest, very slight cough with no sputum. He was not confined to bed and was not seen by a doctor before his death on 28th August. Postmortem findings confirmed by direct smears, microscopical sections, bacteriological and biological tests carried out at this Institute, revealed plague of the septicaemic type. Virulent strain of *S. pestis* was recovered from postmortem material.

Considering the clinical septicaemic form observed in this ambulatory case in the proximity of a gold-mining centre, it was decided to proceed with the immunization of all Native and European populations in the immediate vicinity of this suspect area. From 30.8.43 to 2.9.43 130 Europeans as well as 300 Natives were immunized. For measures of safety vaccination was also extended to the Native labour complement of the neighbouring South Rookpoort Mam Reef gold mine. On 5th September 1943 1 007 Native workers from the above mine were vaccinated. Each Native received 1 c.c. avirulent plague vaccine. Analyses of the mild type of vaccinal reaction observed in the series have been discussed earlier in the paper. No further cases were reported in this area.

Extensive anti plague measures—deratization in suspect urban areas and gassing of rodents in open peri-urban areas of veld were reinforced on the whole Rand. During the year 1943 a total of 11 784 rodents were submitted for examination to the South African Institute for Medical Research. *S. pestis* was found in twenty-one of 774 from various parts of the Union. Five of 11 008 from the Johannesburg municipality. Of sixty three groups of fleas submitted, positive results were obtained from seven.

These energetic measures resulted in a mass destruction of veld rodents and rat populations in several areas, and were followed by the disappearance of plague epizootic on the Rand. Since October 1943 no more plague-carrying rodents have been traced in the daily examinations at this Institute of rodents sent from this area.

#### OVAMBOLAND PLAGUE OUTBREAK (SOUTH WEST AFRICA) AND PLAGUE CONTROL IN THIS TERRITORY BY MEANS OF AVIRULENT VACCINATION

The following data regarding a plague outbreak in this territory in 1943, is taken from information kindly supplied by the CHIEF MEDICAL OFFICER, South-West Africa, and the military authorities of the South West Africa Command, who authorized their publication.

An outbreak of bubonic plague was first reported on 11.1.43 in western Ukuanyama and the eastern district of Ukuambi in Ovamboland. Plague originating from veld rodents has been endemic in this territory during the past 12 years. During the following six weeks, up to 20th February 1943 thirty three cases of bubonic plague, with eleven deaths, were reported. Anti-plague immunization of the Native population of the infected districts was organized by the South-West Africa Public Health authorities and the local military authorities also made arrangements in the Union for anti-plague immunization of all Union Defence Force Native personnel proceeding to Ovamboland, prior to their departure from the Union.

No further bubonic cases were observed among the non-immunized population until early April, when three fatal cases of pneumonic plague occurred in Ondangua area. Seven other pneumonic cases were reported in the

Ukuanyama area, all of them fatal. From January to June, 1944, a total of 201 plague cases were observed with forty-six deaths in the non-immunized Ovamboland Native population.

Type of plague	Cases	Deaths
Pneumonic	10	10
Septicaemic	2	2
Bubonic	189	34

During the same period 6,308 Natives were immunized in Ovamboland, 1,560 with live plague vaccine (one dose of 1 c c) and 4,748 with heated vaccine still in stock (two doses of 1,000 and 2,000 millions). Up to the end of June, 1943, no case of plague had been observed among immunized persons. Disinfection of the Native kraals involved, and other anti-plague measures were enforced by the Rodent Officer of Ovamboland. As a result of these prophylactic measures the outbreak is reported to have subsided since May, 1943, with only a few isolated bubonic cases in June among non-immunized persons. None were of a serious nature and this marked the end of the outbreak. In their control work, the Union Defence personnel in this territory during this plague period under review (January to May, 1943) immunized a total of 522 men, all Natives, with live plague vaccine at the Military Hospital, Tsumeb, before proceeding to Ovamboland. They have declared the position as very satisfactory, as not a single case of plague has been notified among these men. With the co-operation of the Medical Department of the South-West Africa Administration, the following additional steps were taken concerning men returning from Ovamboland —

(a) All details reporting back to Ondangwa from pneumonic plague-infected kraals were given 50 c c anti-plague serum and were detained there for 3 days by the District Surgeon before being sent back to Tsumeb.

(b) Details returning from Ovamboland were medically examined at Tsumeb for symptoms of acute respiratory disease or signs of bubonic plague. These prophylactic plague measures continued to be enforced. From June, 1943, to November, 1944, several thousands of the Native Military Corps were immunized with avirulent plague vaccine at Tsumeb Hospital before proceeding to Ovamboland. No case of plague was observed among the latter. From information supplied by Dr W. CAMPBELL, District Surgeon of Ovamboland, in an interim report (dated 5th December, 1944), over 11,000 Natives were inoculated with live plague vaccine during this subsequent period (26th June, 1943, to 30th November, 1944) in this territory. These included 9,474 local labour recruits and some 2,000 labour recruits sent from Ovamboland to South-West Africa proper. Among the former were 385

Native plague contacts—ten European plague contacts were similarly immunized and 1,534 Natives also received killed plague vaccine. Among these inoculated groups only one case of bubonic plague not fatal, was observed. During the same period, seventy-eight cases of plague, seventy-seven bubonic and one septicæmic, with eight deaths were recorded among the non-immunized Ovambo and Nati population. These epidemiological results in a territory where plague has an endemic character are a gratifying result of the use of prophylactic immunization and of the efficient co-operation taken in the control of plague by civilian and military health authorities of the territory.

#### PROPHYLACTIC PLAGUE IMMUNIZATION IN THE UNION DEFENCE FORCE

In addition to the above case the Union military authorities have used avirulent plague vaccine for the immunization of certain European and Native units either stationed in the Union or belonging to expeditionary forces engaged in war operations in plague infected zones, such as Madagascar. Although military security reasons do not permit the disclosure, at present, of relevant details it may be stated that over 5 000 U D F personnel were immunized with avirulent plague vaccine without any untoward clinical reactions or other reactions affecting their fighting capacity and that no case of plague was observed among the troops so immunized.

#### DISCUSSION

The analysis of fourteen plague outbreaks which occurred in Southern Africa during the period 1941 to September 1944 when avirulent plague vaccine was used, and the prophylactic use of this vaccine among the Army and civilian population, reveal the following facts—

- 1 The harmlessness of the method as shown by the absence of any untoward reactions in a total of over 40 000 persons immunized with a single standard injection.

- 2 The very mild type of vaccinal reactions observed in the various sections of the immunized population, European, Native and Eurasian, both adults and children, including babies under 2 years of age.

- 3 The rapid development and high degree of specific anti plague protection following immunization against the main epidemiological and clinical forms of the disease, bubonic, septicæmic and pneumonic protection is manifest as soon as the 5th day after inoculation. This is shown by the small number of plague cases among immunized persons even among very close contacts of pneumonic cases. As an example, we may cite a case quoted by Dr CLARK (1943) of a Native woman who attended four fatal cases of pneumonic plague in her household. Besides avirulent plague vaccine she also received 100 c.c. anti plague serum prophylactically. In spite of this close and repeated infective contact she did not develop the disease.

- 4 Vaccination was followed in all cases by the termination of the outbreak,

bubonic or pneumonic, in a maximum period of 10 days after the beginning of inoculation

5 Of a total of over 24,000 immunized persons with avirulent vaccine during these fourteen outbreaks of which we have definite records, only fifteen cases of plague have been observed. These include seven pneumonic cases with five deaths and two recoveries, seven bubonic cases with two deaths and one septicaemic case who recovered

6 Epidemiological and clinical particulars of these three categories of cases regarding the relative lapse of time between immunization and infective contact, and of onset of symptoms and death are given in the following table —

Case number	Sex and age	Clinical form of infection	Time of contact with plague case	Time of onset of plague symptoms	Days of illness stated if followed by death
I	Native, F, 70	Pneumonic	3 days <i>after</i> immunization	5 days <i>after</i> immunization	Recovered
II	Native, M., 30	"	3 " " "	5 " " "	2 days
III	Native, M, 35	"	2 " <i>before</i> "	10 " " "	2 "
IV	Native	"	?	Within 8 days <i>after</i> immunization	2 "
V	"	"	"	Within 8 days <i>after</i> immunization	2 "
VI	"	"	"	Within 8 days <i>after</i> immunization	2 "
VII	"	"	?	6 days <i>after</i> immunization	Recovered
VIII	"	Bubonic	Same day or day after immunization	3 " " "	"
IX	Native, M., adult	"	?	8 " " "	"
X	Native, M, 11	"	2-4 days after immunization	During week <i>after</i> immunization	2 days
XI	Native, F 10	"	Contact at least on day of immunization	2 days <i>after</i> immunization	3 "
XII	Native, adult	"	2-4 days <i>after</i> immunization	Within 8 days <i>after</i> immunization	Recovered
XIII	Native, adult	"	2-4 " " "	Within 8 days <i>after</i> immunization	"
XIV	Native, child	Septicaemic	4 " " "	6 days <i>after</i> immunization	"
XV	Native	Bubonic (Ovamboland)—dates not obtainable			"

As shown in the above table, of the six bubonic cases (Cases VIII to XIII) observed among immunized communities, four (Cases IX, X, XII and

XIII) developed symptoms within the 8 days following inoculation. (In three of them, the actual day could not be ascertained.) Of these four cases, one, Case X, died. In this case infective contact took place 2 to 4 days after immunization. In the second fatal case Case XI infective contact probably took place on the day of immunization.

In the only septicæmic case immunized, Case XIV contact occurred on the 4th day after immunization, *i.e.* 1 day at the earliest before protection is expected to be derived from immunization. In spite of its severe clinical form, this case treated with sulphapyridine M & B 693 recovered.

In Cases I and II of the seven pneumonic cases (Cases I to VII) among the immunized communities, infective personal contact took place on the 3rd day *after* immunization followed in both cases by the onset of pneumonic symptoms days later *i.e.*, on the 5th day after vaccination. Of these two cases, Case I, bacteriologically confirmed, recovered and the other died on the 2nd day of illness. In the third pneumonic case of the same outbreak (Case III) personal contact took place 2 days before inoculation. Onset of pneumonic symptoms appeared only 10 days after the latter bringing the incubation period up to 12 days. As stated earlier it is reasonable to infer that the giving of 500 c.c. anti-plague serum as a prophylactic measure had the effect of prolonging the incubation period.

In the other four pneumonic cases (Basutoland outbreak, 1943) the onset of symptoms occurred in each case within the 8 days following immunization. It is regrettable that no exact dates could be ascertained. Of these cases, three died 2 days after the onset of the disease while one recovered without treatment. Although no bacteriological confirmation was possible, according to Dr DILL, Director of Medical Services Basutoland who personally attended the cases, epidemiological and clinical evidence left little doubt about the pneumonic plague nature of the case. While on the subject, we recall a third case of recovery from pneumonic plague observed in South Africa, also following avirulent plague immunization. This case was the first recovery to be reported and was observed by Dr GALE and referred to by us in an earlier publication (GRANET 1942). This case was the last of a series of thirty-seven pneumonic plague cases in an outbreak in 1941 in the Morokwen Reserve in the Kalahari. While six other cases who had been immunized from 1 to 5 days *after* contact with pneumonic cases all died, in this case which recovered vaccination was done 5 to 6 days *before* infective contact with pneumonic cases. According to Dr GALE (1941), she was a girl of 17 who had nursed her stepmother who died with characteristic symptoms of pneumonic plague and was clearly linked with other fatal pneumonic cases. She had received 1 c.c. live avirulent vaccine 5 to 6 days before infective contact. Onset of symptoms appeared 4 days after the latter. She showed similar clinical signs to the other cases who died. Her cough was light but definite on the 4th day there was crepitation at the base of the left lung but no consolidation. Sputum was scanty so that it was

impossible to obtain enough for bacteriological confirmation of the diagnosis, which on epidemiological and clinical grounds could not be doubted. The girl was ill, stuporous and toxic. She was fully expected to die as the others had done. For that reason plague serum was not given until the 4th day, on the morning of which she seemed to rally a little. She was given then 200 c.c. of anti-plague serum. She was distinctly better by evening and eventually after a fortnight of fever, malaise, etc., she recovered completely. Dr. GALE, who attended the case personally, is of the opinion that this case of recovery was due to preventive active immunity derived from vaccination which apparently carried the patient through the crisis for she was definitely better before she received anti-plague serum.

Finally, on reviewing the fourteen plague outbreaks referred to above, it will be seen that no further case of plague, bubonic, pneumonic or septicaemic has been recorded among immunized persons after the 10th day following the institution of immunization. Epidemiological analysis of this field immunization against plague must be considered in the light of the local conditions and circumstances in which it was applied. In all cases avirulent plague vaccination was offered to the whole of the population at risk, European and Native, and was usually accepted by the majority of the population concerned. Close and intimate contacts were immunized practically without exception. No actual non-immunized "control" was therefore available.

Information supplied by the Medical and Public Health authorities in charge of the respective outbreaks for the period prior to immunization, provides us, however, with valuable data regarding the epidemiological character and the evolution of the outbreaks, average period of incubation, clinical forms of the infection, relative severity of the cases and the death-rate among non-inoculated population. These epidemiological and clinical data, together with laboratory investigations, *i.e.*, bacteriological and virulence tests of infected material obtained in most of the outbreaks, give a general picture of the epidemiological conditions in which immunization was introduced and also allows a critical interpretation of the changes which were observed subsequent to immunization.

#### SUMMARY AND CONCLUSIONS

A review of the anti-plague immunization with live avirulent vaccine in Southern Africa, including fourteen plague outbreaks, from 1941 to 1944, with epidemiological and immunological analysis, leads to the following conclusions —

- 1 The harmlessness of the method, as evidenced by the absence of untoward reactions in over 40,000 doses used in preventive mass immunization of European and Native populations during the period.

- 2 The very mild type of vaccinal reaction observed in various sections of the vaccinated communities, civilians (adults of both sexes, children and old people) as well as mining and military organizations. In male adults local reaction was limited to 4.2 per cent and moderate general reaction to 3.7 per cent.



3 The rapid development and high degree of specific protection following immunization. This is shown by the limited number of plague cases, a total of fifteen cases with seven deaths, observed among over 24 000 persons immunized during nine bubonic and septicaemic, three pneumonic, and two mixed bubonic pneumonic and septicaemic outbreaks.

4 Epidemiological and immunological analysis of respective cases, viz bubonic, one septicaemic and seven pneumonic is given, taking into account time relationship between date of immunization, infective contact, onset of disease and eventual death or recovery.

5 Protection derived from immunization begins to be effective from the 5th day after inoculation, reaching full benefit towards the 10th day after inoculation. The latter date has in every instance marked the termination of the outbreak, bubonic or pneumonic. The few cases of plague observed from the 5th to 10th days after immunization usually ran a less severe course, with reduced mortality. These include three cases of recovery from pneumonic plague who developed the infection at this critical phase—one bacteriologically and biologically confirmed and two further close pneumonic contacts without laboratory confirmation for which, however, there was full epidemiological and clinical evidence.

6 Single live avirulent plague inoculation (1,000 million organisms) with usual anti plague measures, realizes a safe, easily applicable well accepted and efficient mass plague control method, in the epidemiological field conditions existing in South Africa.

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## TRANSMISSION OF DENGUE FEVER BY *Aedes (Stegomyia)* *scutellaris* WALK IN NEW GUINEA

BY

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### 1 INTRODUCTION

The writer of this paper is to be considered its author only in so far as he planned and arranged the experiments and collated their results. Most of the actual work was carried out by the several, widely separated groups of workers whose names appear in the appropriate sections of the text. Each submitted a report on his phase of the work, and the writer was instructed to present the results in a single, coherent statement.

The problem with which we were faced was this. Classical urban dengue, transmitted presumably by the ubiquitous *Aedes aegypti*, was well known and not infrequent in the settled parts of the Northern Territory, North Queensland and Thursday Island, and at Port Moresby in Papua. It presented no special difficulties. A similar disease, however, appeared sometimes sporadically, often as an epidemic, in various uncivilized parts of New Guinea remote from any known infestation with *Aedes aegypti*. At first the diagnosis was in doubt, some regarding it as dengue and others as malaria, while still others labelled it as sandfly fever, although no species of *Phlebotomus* which feed on human blood are known from the region. The differentiation from malaria was particularly difficult, as malaria was raging at that time: men were taking suppressive quinine, and sub-clinical trophozoite waves were frequent, no doubt often coinciding with the dengue-like attack. The occurrence of fever with parasites in the blood, would lead to one inevitable conclusion.

These cases, however, did not respond to quinine, and, although the clinical picture varied considerably, as in all dengue outbreaks, the characteristic headache and suffusion of the face and eyes were almost always present, and one could always find some patients with typical saddle back fever, or rash, or both.

\* In addition to those who collaborated in the Lae experiment, and whose names are mentioned in the text, I would thank Lieut-Colonel R R ANDREW, Major M J MACKERRAS, and Captain G A J PASFIELD, for their help in the earlier experiments, while Captain F W BERRILL's biological studies have been freely drawn on. To Acting Professor E A BRIGGS we are indebted for the facilities made available in the Department of Zoology, University of Sydney. Special appreciation is due, too, to the twenty-six volunteers who co-operated so willingly in these experiments.

Finally, our thanks are due to the Director-General of Medical Services, Major-General S R BURSTON, for permission to publish.

The opinion that the disease was dengue fever transmitted by some hitherto unknown vector became generally accepted.

These observations introduced a fresh parallelism between dengue and yellow fever but it is not suggested that it goes beyond the fact that "urban" and "jungle" forms can occur in both diseases. There is no suggestion as yet of any animal reservoir for "jungle" dengue fever; indeed, the evidence, where there is any (e.g. at Milne Bay), favours the view that the infection was brought in by the troops themselves from endemic areas on the mainland, and that it simply found unexpectedly favourable conditions for propagation. It was obviously desirable to discover the vector and assess the possibilities of its control.

## 2. PRELIMINARY OBSERVATIONS.

The known vectors at that time were two —

*Aedes (Stegomyia) aegypti* Linn.—Incriminated by BANCROFT (1906) in Queensland proved by CLELAND BRADLEY and McDONALD (1916, 1918) in Australia by SMYTH, HALL and HITCHINGS (1926) in the Philippines, and others.

*Aedes (Stegomyia) albopictus* Sk.—Incriminated by KOZUMI, YAMAGUCHI and TOKOJIMURO (1917) in Formosa proved by SANDOZ, ST. JOHN and REYNOLDS (1930) in the Philippines and by SNIJDERS, DRYER and SCHÖFFNER (1931) in Sumatra.

*Culex fatigans* Wied. (= *C. quinquefasciatus* auct.) was believed to be a vector by GRAHAM (1903), but it is now known that transmission by this species, if it ever occurs, is only mechanical (cf. SANDOZ, ST. JOHN and REYNOLDS, 1931). KOZUMI *et al.* (1917) also stated that *Armigeres obturbans* Walk. (= *Dessoules obturbans* Walk.) transmitted dengue, but on evidence of distinctly doubtful validity.

The earlier observations in New Guinea were made by the writer in outlying parts of the Moresby district, where infections were occurring, but to which *A. aegypti* had apparently not extended and at Milne Bay where no *A. aegypti* could be found anywhere. The one abundant species which was a pest in both areas was *Aedes (Aede) funereus* var. *ornatus* Theo., but *Mansonia (Mansonioides) uniformis* Theo., *Aedes (Ochlerotatus) vigilae* Skuse, and *Aedes (Aede) similis* Theo. were also common in the Moresby area. *Aedes (Stegomyia) scutellaris* Walk. was not uncommon at Milne Bay but too rare at Moresby for experimental trial and *Armigeres* spp. present in moderate numbers at Milne Bay were absent at Moresby.

Two experiments were carried out. In the first adults of *A. funereus* var. *ornatus*, *A. similis*, *A. vigilae* and *M. uniformis* were collected in the field at Port Moresby fed on cases diagnosed as dengue fever flown to Sydney kept there in a hot room, and fed after interval of 10 to 20 days on clean volunteers. The second experiment was similar except that *A. funereus* var. *ornatus* alone was used. In no instance did the mosquitoes transmit dengue.

The acquisition of Lae and Finschhafen, and the development of these areas as bases, was accompanied by considerable outbreaks of dengue fever, which extended also up the Ramu valley. A survey by Capt F. W. BERRILL (unpublished report) showed that *A. scutellaris* was the most abundant biting culicine, that its local distribution coincided with that of dengue fever, and that its density was roughly proportional to the number of cases of dengue occurring in any particular area. Other pest species, which could not be excluded on epidemiological grounds, were *Armigeres brevipalpis* Taylor, *Armigeres milnensis* Lee, and possibly in certain limited areas *Aedes (Finlaya) kochi* Don. It was therefore decided to carry out an experiment with such of these species as could be obtained in sufficient numbers. At the first attempt, Captain BERRILL successfully fed the mosquitoes and held them for the extrinsic incubation period, but air transport to the mainland failed at the critical time. The second experiment is described in the next section.

### 3 THE LAE EXPERIMENT

#### a SELECTION OF DONORS (Lieut-Col BRUCE HALL)

Seventeen donors in all were used, eight for *A. scutellaris*, six for the species of *Armigeres*, and five (including two also used for *A. scutellaris*) for *Aedes aurimargo* and *Tripteroides* spp. All were seen by two medical officers, who had to agree on the diagnosis of early dengue fever before a man was used for experiment. All but one were used within 48 hours of onset, and most within 36 hours. The exception, a man used to feed *Armigeres* sp., was in the 3rd day of the disease. This man and one other also used for *Armigeres*, had a remission of temperature when the mosquitoes were fed, otherwise all were febrile at the time they were employed.

Clinically, all were typical cases of dengue fever as it was seen in the epidemic then in progress, and in all the diagnosis was confirmed by the course of the disease subsequent to feeding the mosquitoes. The onset was sudden, and the condition was characterized by fever (saddle-back in nine), malaise, aches and pains, headache, post-orbital pain, suffusion of the eyes, palpable lymph glands, palatal vesicles in some, a just palpable spleen in one, and a dengue-type rash in the majority. No malaria parasites were found in any of these patients, and all recovered promptly and completely without specific treatment. Cases with sore throat, cough, or diarrhoea were excluded.

It is to be noted that the men used to feed the different species of mosquitoes were selected at random, and that there was no marked clinical difference between any of the groups. Examination of the charts suggests however, that the *Armigeres* group was on the whole milder than the others, and it also included the two exceptional cases mentioned above. Examples of the charts are shown in Figs 1 and 2.

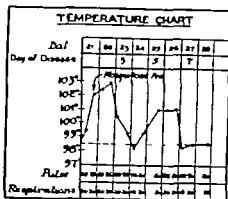


FIGURE 1

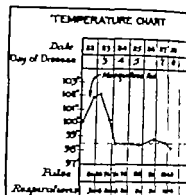
FIG. 1—Chart of Sig. S. used to infect *A. scutellaris*.

FIGURE 2

FIG. 2—Chart of Dyr. K., used to infect *Armigeres* spp.

b COLLECTION FEEDING AND TRANSPORT OF MOSQUITOES  
(Major D. O. ATTHERTON).

As *A. scutellaris* was the species most under suspicion, most attention was paid to it. Larvae were collected in the field, and bred out in the laboratory to form the bulk of the adults used, but wild adults were also collected by tubing them as they came to bite and transferring them to the stock cages. Other species were all collected as adults. Accurate determinations were not attempted at this stage, as it was desired to reduce handling and possible damage to the insects to a minimum. On the basis of hand-lens identification in the tubes, the species collected were divided into four series —

*Aedes scutellaris**Armigeres* sp. 2.*Armigeres* sp. 1*Tripteroides* spp. and *Aedes aegypti*

The stock cages were 9-in. cubes, with wire frames, gauze sides, and a sleeve at one end. To ensure that a high humidity was maintained during storage and transport, the floor of the cage was covered by a pad of cotton wool held in place by a firmly stitched false bottom of gauze. This pad would absorb 2 to 3 fl. oz. of water daily and maintained a satisfactory humidity. Raisins were kept in all cages as food for the mosquitoes. During storage and transport the cages were kept in a plywood container divided into a series of pigeon-holes, the whole being wrapped in a blanket for additional protection. Six cages were used, one for bred *A. scutellaris*, two for mixed bred and field-collected *A. scutellaris*, and one each for the other three series. About 75 to 100 mosquitoes are kept in each cage.

For feeding, the mosquitoes were carried from the laboratory to the hospital in their plywood case. The hand and arm of the patient was inserted through the sleeve and the side of the cage towards the light was kept covered by a towel. Most feedings were done between 11 a.m. and noon, but some between 8 and 8 at night. No special stimuli were used, and all species fed

readily during the day, *A. scutellaris* and the larger *Armigeres* being the most vigorous biters

All species were given at least one opportunity to feed on each of three successive days, a different donor being used each time to increase the chances of infection. Cages A and B (*A. scutellaris*) and D (*Armigeres*) were fed three times, and the remainder five times (three morning two evening). As it was considered desirable to disturb the mosquitoes as little as possible, no accurate counts were made, but it appeared that almost all the mosquitoes (except possibly in the *A. aurimargo* cage) fed at least once, and most of them twice.

Feedings were completed on the evening of 23rd February, 1944. On the morning of 24th February, the case of cages was flown over the range to Port Moresby, duration of flight 2 hours, altitude 10,000 feet, temperature inside aircraft down to about 55° F. On 25th February, the cages were flown on to Brisbane, duration 9 hours (including two stops), altitude to 8,000 feet, temperature down to about 65° F for about 3 hours though not so low during the remainder of the flight. On 26th February the flight to Sydney was completed and the cages delivered to the University, duration 3½ hours, altitude up to 3 700 ft, temperature not below 70° F.

The methods employed seem to have been satisfactory for the mortality up to the time of delivery was not more than about 5 per cent.

### C. MAINTENANCE AND FEEDING AT SYDNEY, NEW SOUTH WALES

(Major A. R. WOODHILL, Mr D. J. LEE)

Immediately on arrival, the mosquitoes were placed in the warm-room, where they were maintained at a temperature of approximately 80° F and a relative humidity of about 80 per cent. Next day, they were identified and sorted into fresh cages.

The species received and the numbers present at the beginning and end of the experiment are shown in Table I.

TABLE I  
MOSQUITOES RECEIVED IN SYDNEY

Species	Numbers present		% mortality
	At 1st March	At 15th March	
<i>Aedes (Stegomyia) scutellaris</i> Walk.	130	67	48
<i>Armigeres breinli</i> Tayl.	19	0	100†
<i>Armigeres mulsensis</i> Lee*	26	4	85
<i>Aedes (Skusea) aurimargo</i> Edw.	44	18	59
<i>Tripteroides bimaculipes</i> Theo.	22	3	86
<i>Tripteroides argenteiventris</i> Theo.	34	16	53

\* = *Armigeres obturbans* var., Edwards, 1924

† = At 12th March.

## d FEEDING AND RESULTS (Lieut.-Col. LORENZO DODD Major P. G. DOWLING)

Feeding commenced on 1st March, when the mosquitoes were approximately 10 days old since emergence or collection in the field.

As only a limited number of volunteers were available for biting, only the first four species were used. The men came to the laboratory so that the mosquitoes were quite undisturbed, except for inserting the arm of the volunteer into the cage each day and the daily collection of dead mosquitoes. They were kept for checking, and the entire contents of the cages were checked at the end of the experiment to verify the identity of all mosquitoes used on the volunteers. The men were allocated in groups of three for each species and used in rotation, all mosquitoes being given an opportunity to feed every day (Table II). It was expected that, with a usual 48-hour interval between feeds,

TABLE II.  
EXPERIMENTAL FEEDS ON VOLUNTEERS.

Species	Volunteer	March, 1944.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Aedes scutellaris</i>	Hay	30	—	—	40	—	—	13	—	—	13	—	—	—	—	—	—
	Trem	—	13	—	—	10	—	—	13	—	—	1	—	—	—	7	3
	Han.	—	—	13	—	—	10	—	—	8	—	—	5	—	—	—	—
<i>Anopheles bromleyi</i>	McL.	—	—	—	1	—	—	5	—	—	3	—	—	—	—	—	—
	Ell.	—	5	—	—	3	—	—	—	—	—	0	—	—	—	—	—
	Fen	—	—	4	—	—	3	—	—	3	—	—	—	—	—	—	—
<i>Anopheles malayensis</i>	Mar	6	—	—	3	—	—	3	—	—	0	—	—	0	—	—	—
	Cle	—	1	—	—	1	—	—	—	—	—	0	—	—	0	—	—
	Boo	—	—	1	—	—	—	—	—	0	—	—	0	—	—	0	—
<i>Aedes aurochryseus</i>	Mun.	0	—	—	0	—	—	1	—	—	0	—	—	—	—	—	—
	Ross	—	2	—	—	—	—	—	—	—	—	0	—	—	0	—	—
	Mill.	—	—	0	—	—	0	—	—	0	—	—	0	—	—	—	—
Days since last insects fed		7	8	9	10	11	1	13	14	15	16	17	18	19	20	21	22
Days since first insects fed		0	10	11	1	13	14	15	16	17	18	19	20	1	22	23	24

Onset of fever

the number of bites received would even out as the experiment progressed, but actually the first man in each group had an advantage which he never lost.

Fifteen young adult male volunteers were employed in this experiment, the age of the youngest being 18 years and the oldest 34. They had not had any recent fever, nor had they previously suffered from dengue. An endeavour was made to select volunteers who had not lived north of Newcastle in order to

obtain subjects who had not previously been exposed to risk of dengue infection. None of those chosen had lived north of Newcastle for long periods, or within 2 months of the commencement of the experiment, though some had been in camp in areas around Newcastle for periods of a few months during a portion of their Army service. The volunteers were selected from a Convalescent Depot, their original admission to hospital having been for operations, such as appendicectomy or tonsillectomy, or following injuries such as concussion or sprained ankle.

On general examination, when selecting the subjects particular attention was paid to the condition of the skin, conjunctivae, buccal and pharyngeal mucous membranes, and to the presence of enlarged lymph glands. In each case, a determination of the blood group, in Tangle test, total and differential leucocyte counts, and an examination of the urine for urobilinogen were performed. Four hourly temperature records and daily examination for evidence of infection were commenced on the 5th day of the experiment and continued in the case of the negatives for at least 13 days after they were last bitten.

The general plan of experiment was to observe each subject for the maximum known incubation period to check any infections that arose by passage to clean volunteers, and then to check the specificity of infections in reactors and the susceptibility of non reactors by inoculation of all experimental subjects warranting it. Only one group reacted, that bitten by *A. scutellaris* (Table III).

TABLE III  
EXPERIMENTS WITH *Aedes scutellaris*

Recipient	Number of bites	Days since infective feed	Results	Cross inoculation	
				Sub-inoculation	Immunity test
1 Hav	82	7-18	+	+	—
2 Han	36	9-20	+	+	—
3 Trem	58	8-23	+	+	

#### CASE 1—R. HAY

First bites, 1st March, last, 10th March. On 15th March, he was febrile (Fig. 3 p. 303), and complained of frontal headache and lower thoracic backache. His subsequent history was as follows—

15.3.44—A faint red macular rash was present on abdomen and chest. A blotchy erythema involved the soft palate and posterior third of the hard palate. The tongue was furred, with prominent red papillae at its tip. Small glands were palpable in both groins. Pain was referred to the back of the neck on flexion of the head.

16.3.44—In addition, he now had pains in the legs and feet, and a few rhonchi were heard at the base of the left lung.

17.3.44—Symptoms improved, signs as before, he now had suffused conjunctivae, and the tip of the spleen was palpable.



18.3.44—The rash was now more extensive, and was seen on the chest, abdomen, back, arms, forearms, hands, thighs and just below the knees. It was not trouble, it consisted of closely set, dull red macules, roughly circular in outline  $\frac{1}{2}$  to 1 inch in diameter. There were some smaller bright red spots which did not appear to be related to the red macules. The palate still showed an erythema and appeared almost purpura. The spleen was palpable.

19.3.44.—The backache was now more intense.

20.3.44.—Backache was now the only symptom. The rash was confluent in some areas, and had faded to reddish brown colour. The palate was almost normal. The spleen tip was palpable. N glands could be felt.

1.3.44.—H now felt well, the skin and palate were normal, spleen not palpable.

Note.—This man had an intermittent urticaria for 3 months prior to experimental illness.

On 16th March blood was taken from Hay and 5 ml. was injected intravenously into Car who had not previously been used for experiment. Four days later (20th March) Car complained of pain on moving the eyes, and had suffused conjunctivae and slightly injected palate and pharynx. His onset was slow and it was 7 days later before he was acutely febrile and had more definite symptoms and a faint rash. His subsequent history was fairly typical, but he failed to show a leucopenia.

On 23rd March, blood from Car was injected intravenously in 5 ml. dose into McL. and Fe. (non-reactors from *Armigere brevis*), and into Hay (Case 1 *vide supra*) and Han. (Case 2, *vide infra*). Hay and Han. remained well indicating that they were immune. McL. had an acute attack of typical dengue 4 days later with sudden onset saddle-back fever, frontal headache, painful eye movements, generalized muscular pains, suffused conjunctivae, injected palate, rash and leucopenia. Fe also had a typical though milder attack of dengue 4 days after injection with one-phase fever but with rash, leucopenia, and other signs and symptoms well developed.

To summarize, Hay developed an acute infection, clinically dengue fever, which was passed serially through two passages by blood inoculation, and to which he was himself immune after recovering from the initial attack.

#### CASE 2.—HAN

First bites, 3rd March. Last 12th March. On 15th March, he became ill, with fever (Fig. 3), headache and lower thoracic backache followed about 5 hours later by pains in the limbs, his history from that time being—

15.3.44.—The conjunctivae were suffused, the palate slightly stippled with erythema and there was faint red macular rash on the back and shoulders.

16.3.44.—The symptoms were still present, he appeared rather drowsy, vomited once, and complained of pains in the ankles. H was tender on pressure over the eyes.

17.3.44.—The pains were less marked, though the backache persisted and he complained of sleeping poorly. H still appeared drowsy and the posterior pharyngeal arch was reddened as well as the palate.

18.3.44.—H complained of slight sore throat, and again had headache and backache. The rash was now more marked on the back from sacrum to scapulae though it was still faint on the chest and abdomen.

19.3.44.—There was now severe frontal headache. The rash was generalized, of the same type as Case 1 and was most marked on back, chest, abdomen, arms, the extensor

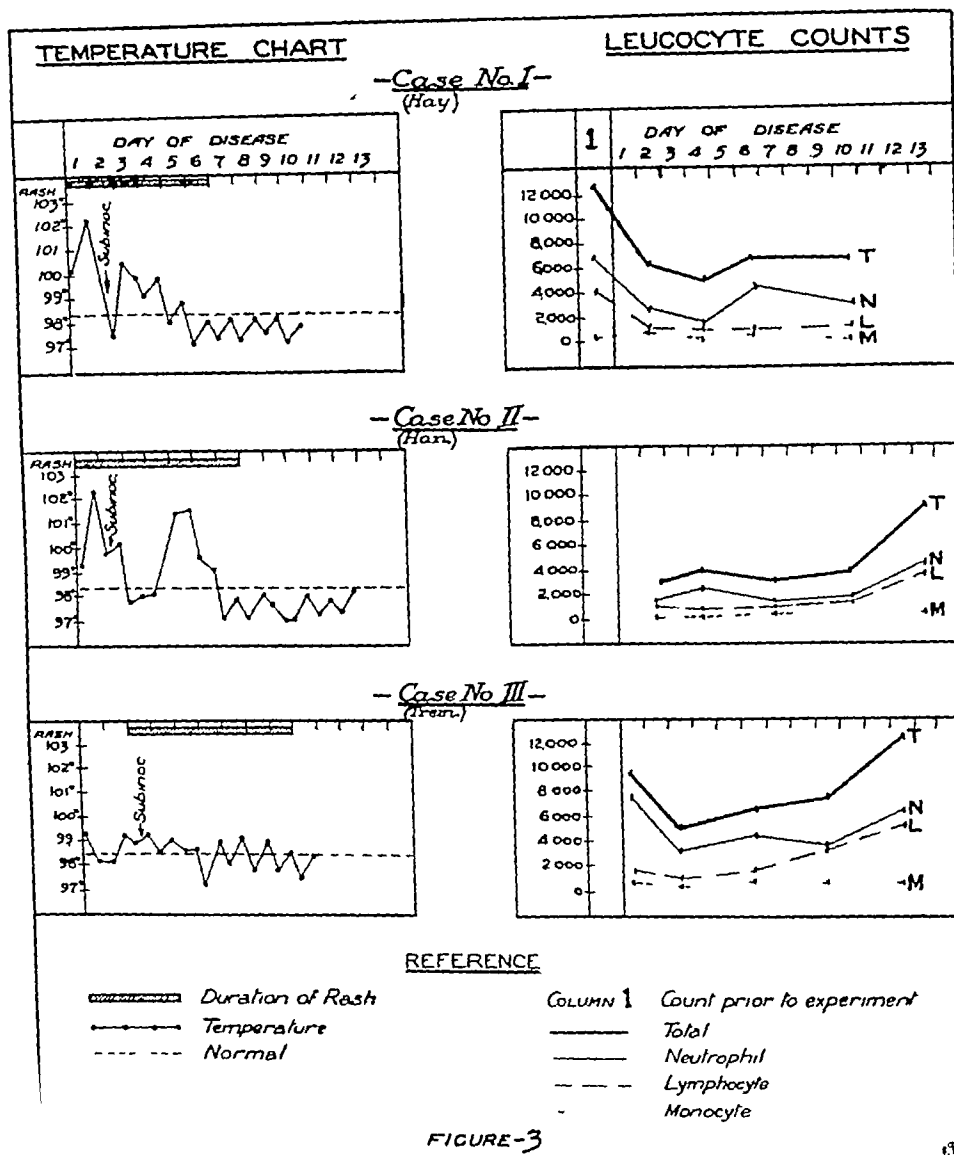


FIG 3—Temperature and leucocyte charts of the subjects bitten by *Aedes scutellaris* [5]

urfaces of the forearms, and the legs. The conjunctivae were clear. The tongue was coated with a white fur with red papillae at the tip. The spleen was just palpable below the costal margin.

20.3.44—The symptoms abated. The rash was fading on the abdomen, but was still present on the back, arms and legs. The posterior pharyngeal wall was clear. The edge of the spleen could still be felt.

21.3.44—He felt well. The rash had almost faded. The spleen was not palpable.

On 16th March, 5 ml. of blood from Han. was injected intravenously into Boo, a clean volunteer who developed a mild attack of fever commencing 4 days later and lasting 3 days. Signs and symptoms however including rash and leucopenia, were well marked and similar to those described in the other cases.

On 21st March, 5 ml. of blood from Boo was injected intravenously into both Ell. (used for *Armigeres bruchi*) and Cle (used for *Armigeres milnensis*). Ell. became ill 3 days later ran a somewhat irregular temperature and showed all the features recorded for the other cases. Cle. had a similar but more severe attack, also after 3 days incubation and he showed a well-marked saddle-back fever. He had mild delirium and muscular twitchings during his recrudescence, and was the most acutely ill of all the experimental cases.

On 23rd March, having recovered from his initial attack, Han. was inoculated with blood from Car (*vide supra*). He remained perfectly well until discharged 11 days later.

Thus Han. like Hay developed a dengue-like infection, which was maintained for two serial passages, and to which he was himself proved to have become immune.

#### CASE 3.—TREM.

First feeds on 2nd March, last on 15th March, onset on 16th March. History (see also Fig 3).

16.3.44.—H. complained of fairly severe frontal headache and lumbar pain, and stated that these symptoms had been present in mild form for 4 days.

17.3.44.—H. now had pains in the limbs especially in the ankles and the right knee joint.

18.3.44.—In addition to the symptoms, there was now sparse, faint, red, macular rash on the abdomen and back. The palate was erythematous, and showed some small vesicles, and the submandibular glands were slightly enlarged and tender.

19.3.44.—There was marked lower thoracic backache, and pain on movement of the eyes. The conjunctivae were suffused, the palate and pharynx injected, and the tongue was coated, with prominent red papillae at the tip. The rash, present on back, abdomen, and chest, consisted of sparse red macules about  $\frac{1}{4}$  inch in diameter. On the extensor surface of the arms and forearms the macules were about  $\frac{1}{4}$  inch in diameter brighter red, and more profuse.

20.3.44.—The symptoms were as before. The rash was now generalized, and involved the palms of the hands and the face. It was least marked on the legs. On the trunk and arms the rash was now maculo-papular in type.

21.3.44.—The symptoms were less severe. The rash was still red in colour and largely confluent on the trunk and arms.

24.3.44.—H. felt well. The rash was fading. The conjunctivae and palate were almost clear.

25.3.44.—The rash had faded, except for some mottling of the skin. The conjunctivae and palate were normal.

On 18th March Smi., who had not been used previously was inoculated intravenously with 5 ml. of blood from Trem.

Four days later he had sudden onset of frontal headache. Next day eye movements were also painful, there was lumbar pain, the conjunctivae were suffused, and sparse faint, macular eruption had appeared on the chest, abdomen, back, and extensor aspects

of the forearms That night he was prevented from sleeping by sharp, intermittent muscular pains in the trunk and limbs His fever was of the saddle-back type, and his subsequent history followed the usual lines

No further passages were made, and an immunity test was not performed on Trem

As the sequence of sub inoculations may appear somewhat complicated from the text, they are set out diagrammatically in Fig 4

Of the negative results little need be said None of the nine men bitten by *Armigeres breinli*, *A. milnensis*, and *Aedes aurimargo* showed any evidence of infection for periods of 13 days or more after the last bites Four of them,

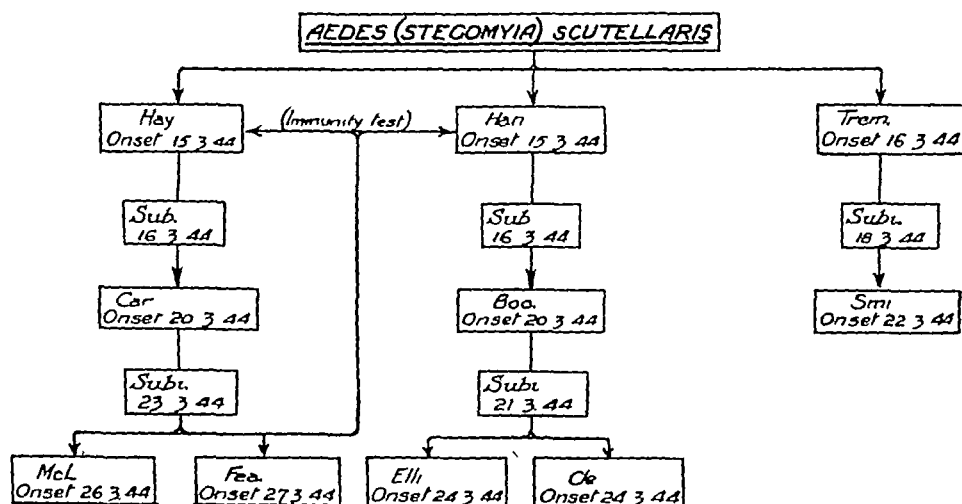


FIGURE - 4

FIG 4 —Diagram representing the passages of the infection used

McL, Elli, and Fea, all bitten by *Armigeres breinli*, and Cle, bitten by *A. milnensis*, were then inoculated with infective blood as stated above. All came down with typical attacks of fever, indicating that they had no pre-existing immunity to the infection.

#### c. REVIEW OF THE CLINICAL FINDINGS (Lieut-Col DODDS, Major DOWLING)

From the case reports, it is seen that Cases 1, 2 and 3, each of which had been bitten by *Aedes scutellaris*, developed an illness characterised by fever, frontal headache and backache, together with pains in the limbs. Each had a generalized rash, conjunctival suffusion, erythema of the palate and a distinctive appearance of the tongue. In each, the fever showed an intermission during its course (Fig 3). In Case 1 this lasted just over 12 hours, while in Cases 2 and 3 it lasted more than 24 hours, though Case 3 had only mild pyrexia at any time. A leucopenia, mainly the result of a marked decrease in the number of neutrophil cells, was observed in each case. The volunteers inoculated with blood taken

from these patients also developed a febrile illness with similar signs and symptoms.

It is considered that the clinical features of the illness in all these cases were sufficiently characteristic for a diagnosis of dengue to be made.

As these ten cases represented a single series, all infected from the same source, some discussion of the clinical findings may be of interest.

*Prodromata.* One case had mild headache and lumbar pain for 4 days before the onset of definite symptoms and signs. Two others had a mild sore throat with pharyngitis for periods of 3 and 7 days respectively but this appeared to be due to an intercurrent infection prevalent at the time.

*Onset.* In one case only the onset could be described as gradual lumbar pain being the only symptom. The other nine cases had a rapid onset, and in two of these, the illness began suddenly. The features most commonly observed at the onset were frontal headache, lower dorsal or lumbar pain, a flushed or puffy appearance of the face conjunctival suffusion erythema of the palate and a macular rash on the trunk.

*Temperature* (Fig. 3). Adopting MEXAW's classification of the types of fever commonly found in dengue (ROGERS and MEXAW 1942), four of these cases exhibited the saddle-back type of chart (HAY SMIL, McL. Ck.), five were of the interrupted fever or two phase type (HAN, TREM., CAR BOO., ELL.), while one was of the short fever or one phase type (Fe.).

*Pulse.* During the febrile stages a relatively slow pulse was a feature of each case, the pulse rate rarely rising to more than 100. Post febrile bradycardia was not observed. The pulse rate had in each case been recorded for at least 14 days before the onset of fever and comparison of pre-febrile and post febrile pulse rates revealed no significant change.

*Pains.* Frontal headache backache in the lower thoracic or lumbar region, and pain on movement of the eyes were early and persistent features in these cases. Frontal headache and backache were each present in nine cases and the eyes were painful on movement in eight cases. Tenderness on pressure over the eyes was noted in six cases. Complaint of pains in the limb muscles was made in eight cases. These pains were generally later in onset than the headache, backache and painful eyes, and their duration was about 4 days. They were referred to the thighs, calf muscles and the regions of the wrists, knees, ankles and feet. The limb muscles were tender on pressure, and there was complaint of pain on movement of the adjacent joints but the pain was referred to the muscles and not to the joints. One case had pain and tenderness in the abdominal muscles. In three cases flexion of the neck caused pain but there was no true neck rigidity. In general the degree of pain varied with the degree of fever those cases having a saddle-back or two phase type of temperature chart having a corresponding remission and exacerbation of pain.

*Rash Exanthem* (Fig. 3). Each case developed a rash and, while there were variations in the extent and intensity of the eruption, many characteristics were common to all.

Faint red macules, roughly circular in outline, sparsely distributed on chest, shoulders, back and abdomen, were first in evidence, and were seen on the first 3 days of the illness. In some cases the rash disappeared after 24 to 48 hours, to be followed by the appearance of more closely set red macules, smaller than the initial macules. This rash appeared first on chest, abdomen and back, then spread to the arms, forearms, palms of the hands, legs and feet, and in some cases to the face. It tended to become confluent, producing a mottled appearance, with areas of normal white skin between the confluent erythematous areas. Over some areas, superimposed upon this rash, were small, bright red spots, about  $\frac{1}{8}$  in in diameter, irregular in distribution, and apparently not related in position to the macules of the general eruption. The rash faded in the order in which it appeared. In some cases the confluence of the macular rash produced a generalized erythema, and it was noted that the two cases which had the most profuse rash showed the least pyrexia, and in these two cases a papular element was present. In two other cases, a slightly raised erythematous eruption, which was very irritable, was present on the hands and feet just before the rash faded. The shortest duration of the rash was 4 days, the longest 11 days. No desquamation was observed in any of these cases.

*Enanthem* An erythema of the palate was present in all, being observed on the 1st day of illness in six cases, and in the remainder by the 4th day. It persisted until after the disappearance of the exanthem in most instances. At first it presented a blotchy or stippled appearance, becoming more confluent in the next 2 or 3 days, though in some cases the stippled appearance became more pronounced, appearing almost haemorrhagic in type. The pharynx showed a similar appearance to the palate in four cases. The tongue was furred in the early stages and later showed a typical appearance, the dorsum being covered with a white fur with large red papillae present over the anterior half inch.

Each of the cases showed conjunctival suffusion. In five this was seen on the 1st day and in all before the 4th day. The average duration was 5 days.

*Lymph Glands* Enlargement and tenderness of lymph glands were not prominent features, though they were present in mild degree in five cases. The groups affected were the submandibular glands, and those in the axillae and groins.

*Spleen* Slight enlargement was seen in two cases only.

*Liver* The liver edge was palpable in one case, while another had tenderness in the right upper quadrant of the abdomen.

*Leucocytes* (Fig 3) All the cases except one showed a leucopenia at some stage. The decrease in neutrophil cells was the main factor in the production of the leucopenia but in most cases the lymphocytes were decreased as well.

*Other Symptoms* Anorexia and constipation were present in all cases during the febrile period. Vomiting occurred in one case and diarrhoea was

present in one only. Sleeplessness was a common feature, and delirium, with muscular twitchings, was seen in one case. Epistaxis was seen in one case, and there was complaint of sore throat in three.

#### 4. DISCUSSION.

The positive experiment would seem to be perfectly clean. The mosquitoes were fed in New Guinea on cases diagnosed as dengue fever and men in Sydney developed a febrile illness after being bitten by them. The men had not been exposed previously to risk of dengue, there were no natural infections at that time within several hundred miles, no other febrile illnesses resembling theirs were occurring in the area and the disease was diagnosed by experienced clinicians who had had extensive experience of dengue fever.

Moreover arial passage by blood inoculation was positive, and the two men tested were immune to reinfection after recovery from the original attack. We may safely conclude that dengue fever was transmitted by *Aedes scutellaris* in this experiment.

This finding adequately explains the occurrence of jungle dengue fever over a wide area in northern New Guinea, for the distribution and prevalence of *A. scutellaris* agree very exactly with the occurrence of the disease. It does not, however, fit the observations in the outlying parts of the Moresby district so satisfactorily. *A. scutellaris* was very rare at the time, and *A. aegypti* did not appear to have extended far into the bush. Possibly it actually may have existed further afield than our surveys would have indicated or possibly the men had more contact with the endemic part of the area than their histories suggested. Generally however the broad picture of *aegypti*-carried dengue in the small points of old established settlement and *scutellaris*-carried dengue over wide area away from civilization would seem to hold fairly well and the same may also apply to *A. aegypti* in civilized Thursday Island and *A. scutellaris* in the adjacent unsettled part of Cape York peninsula.

Two points in connection with the positive experiments remain for consideration, firstly the identity of the vector and secondly the incubation period in the mosquito. As regard the first *A. scutellaris* Walk. (1) differs sufficiently from *A. albopictus* Skuse for the species (or group) identification to be relatively simple. Incidentally no member of the *albopictus* group has yet been recorded from New Guinea. Subspecific determination is however more difficult. *A. scutellaris* was divided by EDWARDS (1976) into a number of varieties, equivalent to subspecies, and these have been raised to specific rank by FARNER and BOHART (1945), who have also added several new forms.

The names of these 18 species have been confused. THEOBALD identified the more western as *Stegomyia scutellaris* (Walk.) and named the more easterly one *Stegomyia pseudoscutellaris*. It is now known that the former is really *albopictus* Skuse so that *scutellaris* (Theob. nec Walk.) becomes a synonym of it and that the latter is the true *scutellaris* Walker: the name *pseudoscutellaris* Theo. surviving for one of its subspecies.

The correct application of the available names to these various forms is not in every instance clear, but Mr D J LEE, as a result of a careful examination of the experimental material, as well as adults of both sexes and larvae from Lae and other parts of New Guinea, has concluded that the subspecies used in the experiment was *Aedes scutellaris hebrideus* Edw as at present recognized. Subspecific identification may appear to be unimportant, in view of the fact that the virus will develop in three quite distinct species of the subgenus, but there are epidemiological indications that the races of *A. scutellaris* present in some of the islands may be poor vectors (McQUEEN, unpublished, PERRY, unpublished).

As regards the extrinsic incubation period in *A. scutellaris*, our data are meagre, but they are worth considering because of their bearing on the negatives recorded with other species. The available information is set out in Table II, and it may be examined by working back from the known incubation periods recorded in man. These have been analysed by LUMLEY (1942), who shows that, for 126 cases reported by SILER *et al* (1926) and SIMMONDS *et al* (1930, 1931) and transmitted either by *A. aegypti* or *A. albopictus*, none appeared before the 4th day and 91 per cent lay between the 5th and 8th days. Adopting this as a basis, one may assess the probable extrinsic incubation period in our mosquitoes as between 13 and 19 days. This is longer than has generally been recorded (11 to 13, and down to 8), and the actual period may lie nearer to 13 than to 19 days. The significant portion is marked by heavy lines in the table.

The possibility still exists that other species may also transmit the infection. Our results, summarized from all experiments in Table IV, have been uniformly negative, but by no means all the experiments were satisfying. On the bases of numbers of bites at the relevant period, the experiments with *Mansonia uniformis*, *Aedes vigilax*, *A. funeraus* var *ornatus* and possibly *Armigeres breinli* would appear to be satisfactory. The negative result with *A. aegypti* may be discounted, because it was an unsatisfactory batch (the only one available at the time), which fed badly on the donors lived poorly and gave only three bites to the two volunteers in the 13 to 19 day period. If one discounts this experiment, however, one must equally discount the results with the remaining species namely, *Aedes similis*, *A. aurimargo* and *Armigeres milnensis*. On experimental and epidemiological evidence, one may doubt whether any species outside the subgenus *Stegomyia* transmits dengue fever but a good deal more work would be needed to settle the matter.

### 5 BIOLOGY AND CONTROL OF *Aedes scutellaris*

*A. scutellaris* in New Guinea may be described as a bush species, which profits by association with man. Its normal breeding grounds are small collections of fairly clean rain water in fallen coconuts from which the copra



TABLE IV  
NEGATIVE EXPERIMENTS.

Experiment.	Species.	Recipient	Number of bites.	Days since infective feed.
Morrisby No. 1	<i>Monomia unifornis</i>	M. Cal.	1	8-17
		C. Col.	37	4-10
	<i>Aedes vigilax</i>	E. C. Lee	23	4-10
		L. Bro.	17	8-17
	<i>Aedes annalis</i>	E. M.L.	4	5-14
		T. R. Bar.	1	14-17
Morrisby No. 2	<i>Aedes fuscus</i>	J. A. Wood.	1	4-10
	<i>annalis</i>	J. Kel.	11	8-10
	<i>Aedes fuscus</i>	A. Wy.	24	9-23
	<i>annalis</i>	J. Tat.	2.	9-23
Lee	<i>Aedes aegypti</i>	G. W. R.	13	9-19
		T. Gods.	9	19-20
Lee	<i>Armigeres brevipalpis</i>	F. J. McL.	11	7-18
		W. J. EL.	8	8-13
		G. J. Fin.	9	9-17
	<i>Armigeres subannalis</i>	W. R. Star.	11	7-15
		N. Cle.	2	8-13
		H. E. Bro.	1	9-11
	<i>Aedes aeneus</i>	E. J. Blum.	1	13-15
		W. L. Rees.		8-10

has been removed, hollow stumps of dead trees, hollows at the bifurcation of the branches of mango trees, fallen fronds of coconut or bread-fruit trees, disused utensils in native villages, and the like. It prefers well sheltered shady situations not exposed to the breeze. It does not breed in heavily polluted water like that favoured by *Armigeres* spp. in coconuts still containing copra, nor was it found by Captain BAXENDEN in axils of taro banana, or *Pandanus*. With occupation by troops, breeding rapidly extends to rusty cans (not clean shiny ones), the tops of drums, tarpaulins, discarded equipment of all sorts, in fact, anything which will hold a few ounces of relatively clear water. The adult population as a result, rises steeply to high levels.

The adults are not house-haunters, their normal resting places (BAXENDEN) being close to the ground in cool, damp well shaded situation under low growing shrubs and bushes, well protected from the wind. They are abundant in untended coconut groves but equally numerous in similar situations in the untouched bush. Their range of flight and capacity to infiltrate appear to be light for units, which practised rigorous adult and larval control.

a margin 200 yards outside their area, remained relatively free of *A. scutellaris* and dengue fever, although surrounded by heavily infested country.

Females appear to enter habitations (tents) solely to feed. Their flight is fairly silent, and they show practically no tendency to settle, usually leaving the tent immediately they have engorged. Occasionally in tents with low sides, from which egress is difficult, some engorged females will rest in dark corners for periods up to an hour. A study was made of the periods of biting activity by recording all specimens entering man-baited tents during the 24 hours. The mean results of 21 days' readings in nine different locations are shown in Fig. 5.

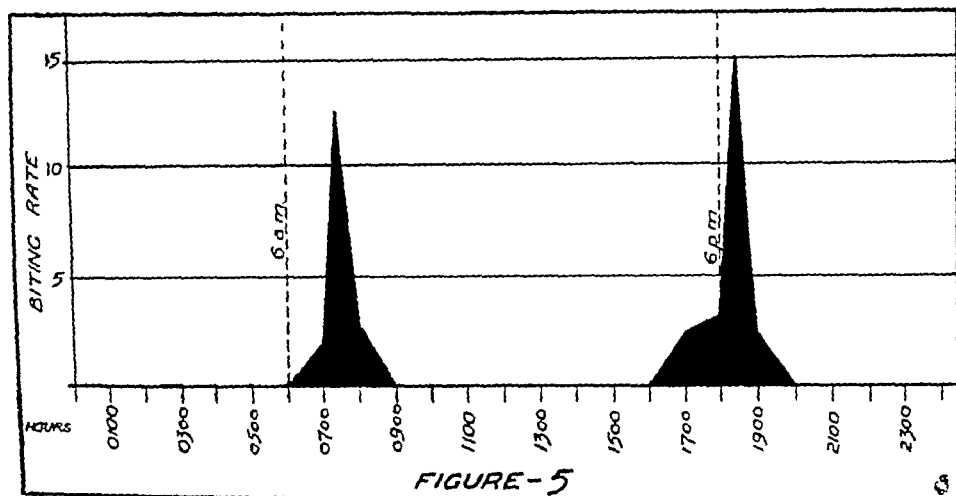


FIG. 5.—Diurnal "biting rates" for *Aedes scutellaris* at Finschhafen, New Guinea (BERRILL)

The rhythm of activity is clearly shown, and remained remarkably constant on different days and in different parts of the area. Occasionally, however, there was a minute accessory peak between 1400 and 1500 hours, and on overcast days, or in tents situated in deep shade, adults would enter and feed vigorously practically all day long.

It is not as easy to control *A. scutellaris* as *A. aegypti*. The same anti-larval measures, namely, clearing up and disposing of all tins and other water-holding rubbish, is the first step to be taken, and is definitely valuable but it must be supplemental by removal of all fallen coconuts and even then residual breeding grounds are left in accessible situations in the bush. The adults are attacked by clearing undergrowth and shelters for a zone up to 200 yards around camps (a procedure which is valuable also against *Anopheles punctulatus*), and by the use of pyrethrum sprays indoors at appropriate times morning and evening. Instructions were issued, too that repellent was

to be used at these times. The results of these measures, vigorously applied, have been good.

The use of DDT against *A. scutellaris* is not yet fully defined. Residual treatment of tents would not appear to be profitable but both adults and larvae are susceptible to attack by broadcast spraying, whether from the air or from the ground. The use of fine drifting mists of insecticide in this way will probably prove the control method of election in the future.

## 6. SUMMARY

1 Experiments are described in which dengue fever was transmitted by *Aedes (Stegomyia) scutellaris hebrideus* Edw fed on patient in New Guinea and on healthy volunteers at Sydney New South Wales. This finding explains the outbreak of jungle dengue fever which have occurred in New Guinea.

2 *Mansonia (Mansonioid) uniformis* Theo, *Aedes (Ochler latus) taylori* Sk and *Aedes (Aedes) funereus* var *ornatus* Theo are probably not efficient vectors.

3 *Armigeres brevipalpis* Tayl. also may not be an efficient vector but the results with *Armigeres nilbrensis* Lec. *Aedes (Aedes) similis* Theo, and *Aedes (Skusea) aurimargo* Edw were inconclusive.

4 The biology and control of *Aedes scutellaris* are briefly discussed.

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## THE AETIOLOGY OF DESERT SORE

BY

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The skin lesion or group of conditions described as desert sore has been reported, from various parts of the Middle East during the war, as having occurred in considerable numbers in British and other troops, particularly in 1941-42

That the aetiology of desert sore (a term that will be applied here to this group of conditions for convenience) is uncertain, is demonstrated by reference to Table I, which summarizes the views held by some writers as to the probable aetiological factor or factors important in its causation

It is our purpose here to describe briefly the results of investigation of sixty-three cases of desert sore seen in Northern Iraq in the period October, 1941, to November, 1942, with a view to determining more precisely the aetiology of the condition, and to discuss the possible aetiological factors involved. The series of sixty three cases consisted of three British officers, and fifty-eight British one Polish, and one Indian Other Ranks, investigated while under treatment in hospital for desert sore or some other condition. Many more cases were seen but could not be fully investigated

### *Terminology and Definition*

Desert sore would appear to be synonymous with veldt sore, Natal sore, septic sore and barcoo rot. It occurred in the Boer War (veldt sore) and in the Great War, 1914-18. A series of cases seen in 1916 in the Sinai Desert was described by CRAIG (1919), and *The History of the Great War* (1924) states that "Septic sores, analogous to the 'veldt sore' of the South African War, were probably the most fruitful source of wastage in the Sinai operations"

Desert sore is a skin eruption, impetiginous or ecthymatous in type, consisting of single or numerous erosions or shallow ulcers, which exude pus and are usually covered with crusts. It affects mainly the exposed parts, tends to extend locally and is slow to heal. If secondarily infected with the *Corynebacterium diphtheriae* the ulcer is deeper, with a punched out appearance and a dark, tough scab

### *Geographical Distribution*

The condition has occurred in desert or mountainous country, in the tropics and sub-tropics, and has been seen in Queensland, N. Australia, Afghanistan and the hot dry areas of India, Iran, Iraq, Syria, Palestine, Egypt, Libya, Sudan, Italian East Africa, South Africa and Gallipoli

\* Acknowledgment is made to Major-General J. G. GILL, CBE, DSO, MC, lately DMS, PAIFORCE, for permitting this paper to be published, to Major J. BOWIE, IMS, and Captain S. C. RAY, IMS, for their help with bacteriological work, and to Captain G. B. ZACHARY, AD Corps, for his report on periodontoclasia. I wish to express my gratitude

In the present series, cases were seen which originated in Northern Iraq (Mosul, Shaqlawa and Qaiyara), Central Iraq (Baghdad and Habbaniya), Southern Iraq (Shuaiba) and in Palestine (Acre and Haifa).

In considering the various places at which the onset occurred the differences in climate should be noted. Wind and dust are excessive at some of these

TABLE I  
SUMMARY OF VIEWS ON THE AETIOLOGY OF DESERT SORE.

Aetiological factor	Authority
Exposure to sun	<i>Mem. med. Diseases in tropical and sub-tropical areas</i> (1942). HENDERSON J. M. (1942).
Contact with animals, especially horses or camels	MANSION-BAKER, P. H. (1940) <i>Mem. med. Diseases in tropical and sub-tropical areas</i> (1941).
Fly-borne infection	MOLESWORTH, E. H. (1937).      RICHARDS, H. (1942).
Sandfly bites	MOLESWORTH, E. H. (1937).
Trauma	MANSION-BAKER, P. H. (1941)      RICHARDS, H. (1942). BITTLEY F. R. (1942).      CHAN, H. S. (1944).
Lack of washing	<i>Mem. med. Diseases in tropical and sub-tropical areas</i> (1941) RICHARDS, H. (1942).
Contagion	BITTLEY F. R. (1942).
Diphtheritic infection	CHANG, C. M. (1919)      MANSION-BAKER, P. H. (1940-1941).
Infection of hair follicles with <i>acrophycoccus</i>	MARTIN, C. J. (1917), quoted by SPECTER, J. H. (1927)
Vitamin deficiency	MOLESWORTH, E. H. (1937). <i>Mem. med. Diseases in tropical and sub-tropical areas</i> (1942).
Lack of green vegetables	<i>History of Great War</i> (1924).      RAPPORT H. M. (1942).

localities slight at others. The difference between winter and summer temperatures is considerable. In addition, the incidence of insects, such as mosquitoes and sandflies, varies greatly.

**Racial Distribution.**—Desert sores were much more common in Iraq among the British troops than among the Indian, and it would appear that men of fair complexion among the former are more prone to the disease. Cases have been seen by me in Iraq and Iran in Poles both in troops and

refugees It was mentioned by RAPPORT (1942) as occurring in Egyptian and Libyan natives, and I have seen one case in an Iraqi man RICHARDS (1943) reported that there was an enormous incidence during the mountain warfare of the Italian East African campaign among British and African troops On the whole it would appear that the races with pigmented skins are more resistant to desert sore

*Seasonal Incidence*—HENDERSON (1943) in the Western Desert found a seasonal incidence of desert sore (most in early- and mid-summer) and puts forward evidence that exposure to sunlight is a factor in its causation BETTLEY (1943) agrees that the incidence is seasonal and that most occur in the summer months in the Western Desert GEAR (1944) in an authoritative paper on Hygiene Aspects of the El Alamein Victory states that its incidence in the Western Desert was highest in the autumn In Iraq however, these sores occurred throughout 1942 During the cold rainy winter in Kurdish Iraq many cases were seen by me and in February, 1942, in one Field Regiment, R A, 23 per cent of the men had desert sores In July, 1942, in the same part, 108 cases were found in a British Infantry Battalion (about 13 per cent), and in October, 1942, in one Light Anti Aircraft Battery of 238 men examined by me, 29 per cent had desert sores

*Local Distribution*—In Iraq it was found that when desert sores were seen in a unit they appeared to be uniformly distributed throughout that unit, i.e., they were not confined to one platoon or company in a battalion Moreover, in any area where desert sores were appearing in a unit, other neighbouring units (British) would be found to be affected also

*Age*—No significant relationship between age and incidence could be found in the series, the ages varying between 21 and 38 (mean 27.5)

*Sex*—All my cases were male and I saw no desert sores among QAIMNS sisters and VAD nurses The proportion of these to male army personnel is, of course, small In Australia barcoo rot is said to occur much more commonly among men than women (MOLESWORTH, 1937)

*Occupation*—Soldiers under conditions of war seem prone to develop desert sore in certain localities The military occupation of the men in this series was as follows—nineteen gunners, sixteen infantrymen seven drivers, five cooks, three fitters, two sappers, two mess waiters and one each of the following—infantry officer, staff officer, medical officer (experimental sore) predictor operator hygiene orderly, signaller stretcher bearer armoured, batman

The number of cases occurring in respect of each occupation is of little significance unless the proportion of men in the occupations in the units of the Army and the R A F served by the hospital is considered However, it would appear that the number of gunners drivers cooks and fitters is disproportionately high—all occupations in which abrasions or burns of the skin are common, and the absence of clerks from the list will be noted

*Site*—The exposed areas of the body are most commonly affected. The incidence in this series was as follows.—The dorsum of the wrist and hands (116 lesions), the anterior aspect of the leg (85 lesions), the extensor surface of the forearm (55 lesions), the front of the knee (36 lesions), the extensor aspect of the elbow and the ankle and foot (26 lesions each), the buttocks (24 lesions), the upper arm (19 lesions), the head and neck (18 lesions) and least commonly the trunk (10 lesions). From about March to November shorts and shirts with rolled up sleeves were worn during the day.

*Onset*—(1) *Primary Traumatic Lesions* Delayed healing is usually observed. The surrounding skin becomes reddened. If the lesion occurs at the site of an abrasion the latter suppurates, if following an insect bite a pustule appears, if at the site of a traumatic blister this becomes filled with pus, and if following a burn the lesion varies according to the degree of burning. Thus a blister becomes purulent or a third degree burn fails to heal the raw surface discharging pus.

(2) *Secondary Vesicles* are usually pinhead in size when first seen. They rapidly increase and within a few hours are 3 to 6 mm. in diameter. During this period they become pustular and are surrounded by a zone of erythema. Occasionally they enlarge to as much as 25 mm. in diameter becoming bullous. These secondary lesions may occur a few inches from the original lesion or on some distant part of the body. For example pustules may appear on the right knee some days after a sore has become established on the left forearm at the site of the abrasion. The pustules usually burst in 1 to 3 days.

*Ulcer Stage*—The lesions, whether following an abrasion, burn, bite or blister or occurring as secondary pustules becomes similar in appearance after about 5 days. A shallow suppurating ulcer with a flat base covered with yellow or greyish debris, and a slightly raised margin surrounded by a zone of erythema, is seen. The ulcer usually increased in size, becoming about 6 to 25 mm. in diameter occasionally as large as 50 mm., and is roughly circular in shape and cyanotic in colour. The margin of the ulcer can be seen to be undermined in the active stage, and pus, thin and yellow or greyish can be pressed from under it. In untreated ulcers there appears to be a natural tendency to heal, but this is usually slow and it may be a month or more before the margin becomes flattened and epithelialization commences.

*Crust Stage*—If no dressing is applied a crust of dried secretion appears over the ulcer in 1 or 2 weeks, and the lesion takes on an ecchymatous or less frequently an impetiginous appearance. The crust is thick, the size of the ulcer and increasing with the latter. (The crust of the only diphtheritic sore in this series was black, smooth and flat, filling the depth of the ulcer its surface level with that of the surrounding skin.) The crust is firmly attached to the ulcer and pressure on it during the active stage will result in the appearance of pus at the margin. Removal of the crust reveals an ulcer as described above. During the chronic stage which may last 8 weeks or more, no further increase

in size takes place, but suppuration is still present and a zone of erythema round the lesion can still be seen. When healing starts the surrounding erythema fades, the margin of the crust becomes loose, and when complete, the crust drops away, leaving a scaly or a smooth scar. This stage of healing may take from 3 to 6 weeks or more.

The scar is seen to be thin, with a glazed surface, often wrinkled, with puckered edges, and in colour darker than the neighbouring skin, being red or maroon. It has a texture rather like that of thin paper, and is without hair or sweat glands. The formation of a scar does not necessarily indicate complete healing, as infection may still be present under the scar, from the edge of which a bead of pus may be squeezed out. Such a scar is surrounded by a zone of erythema and often has a scaly surface. Three scars observed were anaesthetic to pin-pricks. All the other scars, however, were hypersensitive to such pricks. Anaesthesia of the skin round the lesion was only found in three cases.

**Symptoms**—The patient does not usually complain of ill-health, though he may say that he is a little run-down. He often states that whereas he "used to have good healing flesh, cuts now take a long time to heal." The pustule is painful and the ulcer in the acute inflammatory spreading stage is tender and sensitive to pressure and knocks. The ulcer in the chronic stage, and when crusted, is painless. Itching is not present except occasionally when healing is almost complete.

**Complications**—In four cases lymphadenitis was present secondary to the sore (in three cases in the inguinal region, in one in the axilla). One of these cases developed an inguinal abscess which required drainage. Two of these cases also had lymphangitis.

**Bacteriology**—Table II shows the bacteriological findings with the number of cases in which various combinations of bacteria were found.

Fifty one cases were examined by smear and culture, pus from a fresh lesion in the bullous or pustular stage being used when possible. It will be seen that staphylococci were the organisms most commonly seen, being present as *Staphylococcus aureus*, *S. albus*, or as unspecified staphylococci in all but four cases. (Examination of a further eighteen cases not in this series showed that staphylococci were present in sixteen, non-virulent *C. diphtheriae* in one and streptococci in one.) In two cases diphtheroid bacilli were found in the lesions. Virulence tests could not be carried out in these cases. In another case, however, a pure culture of *C. diphtheriae* was obtained and a virulence test was positive (guinea pig).

**Concurrent Disease**—Twelve of the men were admitted to hospital with the following disease in addition to their desert sores.—Benign tertian malaria (three fresh infections), fresh sub-tertian malaria (one), pharyngitis with pyrexia (two), follicular tonsillitis (two), diarrhoea (two), infective hepatitis (one), and with a feverish upper respiratory tract infection. While in hospital, being



treated for their sores, two men developed B.T. malaria (infected before admission), one infective hepatitis, and one a feverish upper respiratory tract infection. It has been stated that malaria is a predisposing cause of yeldt sore. The six cases mentioned above all contracted malaria after their sores had appeared. No case was seen in which the onset of sores followed an attack of malaria.

TABLE II.  
BACTERIOLOGY

Number of cases.	Unspecified staphylococci.	Staph. aureus.	Staph. albus.	Streptococci.	Diplococci (Gram-positive).	Diphtheroids.	C. diphtheriae.	L. tropica.
12	+			+	+			
9			+					
8		+						
6			+	+				
4		+		+				
3	+	+	+					
3				+				
2				+				
2		+		+	+	+		
2			+	+				
1							+	
1								
81	14	17	19	30	14	2	1	0
Total	Number of times organism found.							

*Syphilis*.—No history or clinical evidence of syphilis was present in any men in the series. The Kahn test was carried out in four cases and found to be negative.

*Periodontoclasia*.—Evidence of the presence or absence of nutritional deficiency was sought. Fifty-eight men in this series were therefore examined by Captain G. B. ZACHARY A.D. Corps, with reference to periodontoclasia. He reports —

"Systemic periodontoclasia is the Type II periodontal disease described by BORRIS (1935). In the cases under observation it was diagnosed (a) clinically by changes in the colour and consistency of the gingival tissues—pale, bluish, hypertrophic, with little tendency to pocket formation and (b) radiographically by destruction of the cortical layer as alveolar absorption and thickening of the periodontal membrane. A case of suppurative periodontoclasia is almost indistinguishable from case of systemic periodontoclasia with pocket formation. In the investigation of these cases, therefore, any patient showing clinical and radiographic picture of the systemic disease associated with malocclusion or calculus formation was classed as negative. Positive findings have been confined

to those cases showing typical changes without any evidence of local irritating factors. As the disease leads inevitably to an inability of the dental supporting structures to withstand occlusal strain, early radiographic evidence of alveolar rarefaction and absorption is best seen in the incisor region, where the occlusal force is directed outwards as well as vertically. X-ray examination was therefore confined to the incisor region. Seventeen cases were diagnosed clinically and of these, nine were confirmed radiographically. A further three cases, doubtful clinically, were found by X-ray to be positive. Radiographs could not be taken in all cases owing to shortage of film, so that of twenty-six cases found clinically to be negative, only nine were confirmed as negative radiographically and some of the remaining seventeen might, had they been examined, have shown early radiographic periodontoclusia. Twelve cases were partially or completely edentulous in the incisor region and excluded, and five were not examined. Of forty-six men, therefore, twenty (43 per cent) were suffering from periodontoclusia (systemic). Fifty men, all apparently healthy, and unselected, were examined as controls. Of these, nine (18 per cent) were suffering from clinical periodontoclusia."

The significance of these findings is discussed later.

#### CONCURRENT SKIN DISEASE

*Impetigo contagiosa*.—Nine men (16 per cent) were suffering from this condition in addition to their desert sores. It is possible that the aetiology of these two conditions is similar in many respects, for example, incidence, bacteriology and parallel response to similar treatment. HENDERSON (1943) found coincident impetigo in one in four of his series. BITLEY (1943) considers that impetigo and desert sore may be identical conditions. Of thirty cases of desert sore seen by him eighteen (60 per cent) had impetigo of the face.

*Sycosis barbae* was present in one case and *proriasis* in another. *Prickly heat* was seen in one man.

*Seborrhoea of the Scalp*.—Forty-one men (65 per cent) had a seborrhoeic infection of the scalp with scurf. The typical small greasy scales were present in all these cases and in several greasy crusts in addition. This incidence of seborrhoea is the same as that found in fifty unselected men in hospital for other conditions and is much higher than that seen by me in troops in England early in the war.

*Tolpilia hyperkeratosis* of the buttocks was seen in fifty-three cases in this series (84 per cent).

*Scleritis*.—One patient had scabies immediately before the onset of desert sores. When seen by me the former was cured but had I think been a factor in the causation of his sores, which originated on the buttocks and spread later to the arms (but not the wrists) and legs. Another patient had severe scabies the secondarily infected lesions of which on the wrists, fingers and ankles became typical desert sores.

count for the sixteen cases was 10,000 leucocytes. The differential white cell count revealed no significant variation from normal. The average red cell count was four and a half million (3.0 to 5.5 million).

#### ASCORBIC ACID SATURATION TESTS.

The test dose method, described by AMBART HARRIS and MARRICK (1935), HARRIS and RAY (1935), AMBART and HARRIS (1937) and HARRIS (1942) for estimating the vitamin C level in human beings, is accepted by most authorities as reliable. The method in brief is as follows: standard test doses (700 mg. per 10 stone of body weight) of ascorbic acid are given daily at 1030 hours; at 1400 hours the subject urinates and discards the specimen, and at 1615 hours the bladder is again emptied and this  $\frac{1}{2}$  hours specimen collected and examined for its vitamin C content by titration against 2, 6-dichlorophenol-indophenol. In assessing results the days are counted until about 50 mg. or more of vitamin C are passed during the specified  $2\frac{1}{2}$  hours period, this marking the lower limit for full saturation. Subjects still failing to reach 50 mg. on the 2nd day of the test dosing are described as below standard and each subsequent day beyond is counted as further degree of subnormality. In fully developed scurvy about 7 to 10 days may be needed before saturation approaches completion. This figure gives us a measure by which to judge the extent to which any given subject has dropped on the path toward scurvy (HARRIS, 1942).

Eighteen men in the series were given test doses in this way but supplies of ascorbic acid were not sufficient to complete saturation in every case. The results are shown in Table III. It will be noted that only one man showed response on the 1st day. He had been given 50 mg. of ascorbic acid daily by mouth for 5 days previously. Three men showed response on the 2nd day (but two of these had been taking 50 mg. of ascorbic acid daily for 30 days). Fourteen men required 3 or more days before saturation was attained and were therefore below standard. Nine men without desert sores were also examined (two healthy men, one with malaria, three with whitlows, one with amoebic dysentery, one with multiple abrasions and one with a boil on his face). Of these, one took more than 3 days, three took 4 or more days, and five took 5 or more days, in which to become saturated.

#### DIFFERENTIAL DIAGNOSIS.

Desert sores may be distinguished from oriental sore by their appearance and by the absence of *Leishmania tropica*. *Ulcus tropicum* is a disease of the more humid parts of the tropics but I have seen cases in S.W. Persia (a hot, dry country) among Polish evacuees in 1943. It is a phagedaenic ulcer usually on the legs, and diplococci, spirochaetes (*T. schaudinnii*) and fusiform bacilli can usually be isolated. Desert sores on the legs may be similar to (and sometimes a form of) varicose ulcer. In doubtful cases the Wassermann reaction can be employed to eliminate syphilis. Cutaneous diphtheria may be superimposed on desert sore with a typical appearance as mentioned elsewhere.

#### TREATMENT

Some desert sores clear up without treatment, but only after many weeks, unless the individual can change his environment. In assessing the value of treatment this must be borne in mind. Thus, if treatment is carried out in hospital it may appear effective though the improvement may be due to rest, freedom from trauma and a change in diet.

TABLE III  
RESULTS OF ASCORBIC ACID SATURATION TESTS

TABLE III  
RESULTS OF ASCORBIC ACID SATURATION TESTS

Name.	Age	Weight in stones	Amount of ascorbic acid in test specimen of urine—mg								Number of days required to saturate	
			Day of test dose									
			Resting	1	2	3	4	5	6	7		
T J C*	26	10	14 0	83 2	—	—	—	—	—	—	1	
V V P†	27	11	4 5	42 0	88 1	—	—	—	—	—	2	
D†	23	10	—	45 0	53 2	—	—	—	—	—	2	
G W H	29	10	2 5	5 1	59 6	—	—	—	—	—	2	
S T A.	34	12	—	26 2	30 5	45 4	—	—	—	—	3	
H M B†	22	11	3 5	44 2	38 0	77 4	—	—	—	—	3	
K A J B	27	13	—	6 5	8 4	19 1	—	—	—	—	>3	
D McC	35	9	—	7 4	3 7	3 1	—	—	—	—	>3	
R P	23	10	3 2	3 1	5 6	20 7	105 9	—	—	—	4	
H S	22	11	—	3 4	3 5	5 6	4 5	—	—	—	>4	
A Bu	29	11	—	4 3	4 0	6 8	20 2	—	—	—	>4	
G B	26	14	11 0	6 8	4 9	11 5	9 6	—	—	—	5	
H J B	27	12	2 4	7 4	4 7	11 9	13 5	99 4	—	—	>5	
E P A	37	10	3 7	4 2	5 5	4 7	4 7	5 8	—	—	>5	
A E P	22	11 5	2 0	2 7	3 1	4 7	25 5	40 8	—	—	7	
E W C	25	11	3 4	4 5	3 9	4 0	5 4	8 5	28 4	62 6	>7	
A. B <sub>1</sub>	21	10	5 3	5 2	8 4	6 7	13 5	12 7	12 0	27 7	>7	
F D			6 1	2 5	3 2	4 8	4 5	4 2	6 8	7 7	>7	

ascorbic acid (50 mg daily) taken for 5 days previously  
ascorbic acid (50 mg daily) taken for 30 days previously

\* Ascorbic acid (50 mg daily) taken for 5 days previously  
† Ascorbic acid (50 mg daily) taken for 30 days previously

### Local Treatment

The most important local treatment is rest on the hand or wrist a splint is advantageous. If the desert sore is situated on the leg, rest in bed is beneficial. The number of applications advised for local treatment in desert sore allows some estimate of their ineffectiveness. Sulphonamide powder should not be used as many cases of desert sore treated by daily application have become sulphonamide sensitized. Some authors (MANSON-BAHR, 1940, SEQUIERA, 1927) advise the use of anti diphtheritic serum (4,000 units) subcutaneously in the vicinity of the sore, but this seems irrational unless the *C diphtheriae* has been isolated.

### GENERAL TREATMENT

**Diet**—MOLESWORTH (1937) states that plenty of fresh fruit and vegetables should be given.

**Vitamin Supplements**—The treatment of desert sores by vitamin supplements would be sound if nutritional deficiency, with vitamin deficiency,

was a factor in their causation. It was decided to investigate the effect of vitamin supplements in the present series, and about half the cases were treated in this way and the remainder by local treatment only. For this investigation only fifty-one cases in this series could be used owing to the shortage of vitamins, and as cases discharged from hospital before cure and cases admitted in the dry healing stage were excluded. Only cases with active suppurating lesions were taken into consideration. The results of treatment of these fifty-one cases are summarized in Table IV.

Of the cases on vitamin C, fourteen men underwent ascorbic acid saturation

TABLE IV  
RESULTS OF TREATMENT

Vitamin treatment.					Local treatment.			
Vitamin given.	Number of cases in group	(1)	(2)	(3)	Number of cases in group	(1)	(2)	(3)
C	6	3 to 17 10.8	4.6	20.2	1	3 to 18 6.3	2.0	31.4
C	6	21 to 29 23.0	4.0	12.1	6	21 to 29 23.0	3.7	4.1
C	7	33 to 42 37.1	6.4	4	8	30 to 71 49.6	7.6	41.2
A + C	8	1 to 42 20.6	4.0	17.8				
A	4	7 to 42 22.2	2.5	18.7				
Total	29		Mean healing time 18.6 (days)	23			Mean healing time 31.2 (days)	

(1) Number of days from onset of sores before treatment started and the mean time for the group. Cases roughly grouped for comparison by this mean figure.

(2) Mean number of suppurating sores at onset of treatment.

(3) Mean number of days of treatment before cure.

tion tests and were partially or fully saturated within a few days of commencing treatment. They continued on daily doses of 50 or 80 mg of ascorbic acid, the dose given to the remainder (non-saturation tested) from the start. The quantity given was limited by the short supply. The cases on vitamin C alone received a total each of between 500 and 5,900 mg (Mean total per man 2,740 mg). The cases on vitamin A were given capsules (Crookes) each containing 33,000 i.u. and those on vitamin A alone had a total of 198,000 i.u. each. Those on A and C had a mean total dose of 217,800 i.u. and 2,250 mg respectively. No local treatment, not even a dry dressing was given.

The sores on local treatment were painted daily with brilliant green, 2 per cent in spirit, (twenty cases), or powdered daily with sulphonamide (three cases) as the danger of sensitization was not then appreciated (1942). Splints were used when required, and all affected parts were rested. With one exception (C.F.) both groups were in beds in the same ward, with the same nursing attention and the same diet. Cases were placed alternately in one or the other group on admission. One man (C.F.) having a virulent diphtheritic infection of his sore, was treated with diphtheria antitoxin locally and intramuscularly.

*Two illustrative cases*—J.L.S. and R.I.E., both aged 20, and L.A.C. in the same squadron, R.A.F., had recently arrived together in N. Iraq from Palestine. J.L.S. was an armoured, R.I.E. a ground gunner. They were admitted together to hospital on 20th October, 1942, suffering from desert sores. J.L.S. had ten lesions, the oldest being of 6 weeks' duration, and R.I.E. had six lesions, the oldest being of 8 weeks' duration. The size, stage, situation and severity of the lesions in each case was similar, but R.I.E. had six suppurating lesions and J.L.S. four, the remainder being scabbed and dry. Bacteriological examination revealed no *C. diphtheriae* or *Leishmania* in either case. In both *Staphylococcus albus* was found, and in R.I.E.'s lesions short-chain streptococci in addition. J.L.S. was given 80 mg of ascorbic acid daily with no local treatment (not even dry dressings) from the day of admission. By 25th October, 1942 (in 5 days) all lesions were dry except one new pustule. On 10th November, 1942, after 21 days' treatment, healing was complete. R.I.E., on the other hand, was treated, from the day of admission, by the application of brilliant green (2 per cent in spirit) to the lesions twice daily, and splinting of the right hand (on the dorsum of which was a desert sore). Healing was complete on 26th November, 1942, after 37 days. The diet of both men, while in hospital, was similar.

It will be noted that the mean healing time of the series on supplements of vitamins C, A, and a combination of both was 18.6 days, and of the series on local treatment 31.2 days. The following method of assessing the significance of these results was used—The difference between two proportions is significant when it is greater than twice the Standard Error of the difference (BRADFORD HILL, *Medical Statistics*). The Standard Error of the difference between two proportions is given by—

$$\sqrt{\frac{Pq}{n} + \frac{P_1 q_1}{n_1}}$$

where  $P$ ,  $P_1$  are the proportions in the two samples possessing a character  $q$ ,  $q_1$ , the proportions not possessing that character, and  $n$ ,  $n_1$ , the number of observations in the series.

The main healing time of all the 51 cases was 23.4 days. The number of cases healed in 23 days on vitamins was 22 (P), and on local treatment (P). The number of cases not healed in 23 days on vitamins was 6 (q), and on local treatment 16 (q). There were 28 cases (n) on vitamins, and 23 cases (n) on local treatment. The standard error of difference is therefore —

$$\sqrt{\frac{22 \times 6}{28} + \frac{7 \times 16}{23}}$$

equals 3.10 approx. The difference between the numbers of cases healed in 23 days is 15 (22 - 7) and is much greater than twice the standard error of difference and is therefore significant.

A larger series of cases would be more informative. It must be remembered however that both groups were benefiting by a diet containing more vitamins than that consumed before admission thus tending to lessen the difference between the groups. The evidence suggests that the desert sores in this series were benefited by vitamins A and/or C.

#### DISCUSSION.

Consideration of this series of cases leads to the conclusion that, of the aetiological factors shown in Table I only trauma of the skin and nutritional deficiency appear to be of primary importance. Cases of desert sore occur when the desert is neither dry nor hot, when men are in contact with neither horse nor camel, when washing facilities are good, when sand-flies are absent, and when exposure to the hot sun can be excluded (Case S.T.A. was a man carrying out all his work 60 feet underground and another man a N.A.A.F.I. shop assistant, was indoors all day).

**Trauma**—It would appear that trauma of the skin is an important factor in the causation of desert sores. Abrasions, insect-bites, burns or friction blisters have been the precursor of most, if not all, primary sores in this series. Exposure to sun may be another traumatic factor in some cases as HENDERSON (1943) has pointed out. Desert sores are present more commonly among individuals engaged in occupations in which such trauma is likely to occur than among protected groups such as clerks and women, and they appear on surfaces exposed to trauma. The different incidence among men and women would suggest that desert sore is not as believed by some, an insect-borne disease, although an insect bite, as mentioned above may be the precursor of a sore by causing trauma.

Why does trauma of the skin under certain conditions produce desert sore, whereas similar trauma at other times is followed by normal healing? Just as ecthyma occurs in undernourished children in the United Kingdom, so nutritional deficiency may play a part in the aetiology of desert sore.

*Nutritional deficiency*—The literature contains some evidence that nutritional deficiency may be a factor in the causation of desert sore. *The History of the Great War* (1924) states, in reference to septic or veldt sores

"It was found that the slightest scratch quickly developed into an intractable ulcer which, in spite of all treatment, would last for months, and nothing seemed to do any good except to send the patient down the line to Egypt, when, with fresh food and vegetables and ordinary simple dressing, the sores quickly healed"

Furthermore MOLESWORTH (1937) pointed out the importance of diet in the causation of Barcoo rot. He wrote

"There is reason to believe that unsuitable food or vitamin deficiency plays a part in the determination of sores of this type. Barcoo rot almost invariably affects men living for months in the arid parts of Central Australia. Fresh vegetables and fruit are generally unprocurable in these areas and salted meat or tinned foods constitute the major portion of the diet. Patients suffering from Barcoo rot which has resisted all treatment under the pioneering conditions in which these men live, on being brought to the more settled districts nearly always get well without delay under mild antiseptic dressings. The change from intractability is apparently due to the improved diet which is available."

Certain evidence suggests that nutritional deficiency may have been present in the cases under consideration. The theoretical analysis of the diet has shown that the intake of the vitamins A, B<sub>1</sub>, and C, while not below the minimum thought by many authorities to be essential, is not as high as the optimum. Experience has shown that it is necessary to keep the vitamin intake (theoretical) well above the minimum estimated requirements to prevent symptoms of deficiency, partly because of the considerable loss of some vitamins in cooking, particularly vitamin C (OLLIVER, 1940), and partly because of non-consumption through waste—or distaste. The annotation entitled "Vitamin C Requirements of Man" (*Brit Med J*, 1942) states

"The most generally accepted estimate of the daily requirements of vitamin C by the adult is 50 to 60 mg daily, and much more in both chronic and acute illness and probably in wound healing. But it is most important to realize in making estimates of the vitamin C content of foods that often about half of this factor is lost in washing, soaking and cooking the vegetable in water."

This is borne out by the results of the ascorbic acid saturation tests in this series, and by the findings of DEMOLE (1941), who showed that of ninety-four Swiss soldiers after 9 months' service on a daily intake of 68.5 mg of vitamin C, 57 per cent. were deficient.

The rations of the British Army in the Field have been carefully thought out and are of excellent quality, but the above points should be borne in mind when considering the paper by GEAR (1944) who believes that vitamin deficiency was not a factor in the many cases of desert sore seen in the Western Desert. He points out that "on occasion troops on fresh rations have shown a high incidence [of desert sores], while conversely troops on the so-called 'hard scale' have frequently been free from the affliction."

Before this statement can be considered as evidence against the hypothesis that nutritional deficiency may be a factor in the aetiology of desert sore, it is



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necessary to show that the actual vitamin intake of the men on fresh rations was adequate, and that they were not at a low nutritional level from previous

hard rationing. Furthermore, as regards the absence of desert sores in some men on hard rations it must be remembered that all the members of a community on a deficient diet do not show manifestations of deficiency or the same manifestations. Of Lord Anson's men, when he sailed round the world, a proportion escaped scurvy.

In this series, evidence of the presence of nutritional deficiency (other than the results of saturation tests) was found. That 43 per cent. of the men had systemic periodontoclasia may be significant. Nutritional deficiency is thought to be the chief, if not the only factor causing the condition, and BORTL (1937) wrote that vitamin C deficiency is the only nutritional deficiency which in our experience produces the characteristic features of the systemic type of pyorrhoecia in experimental animals. The same author (with BASSER and WOLBACH) states that changes in the alveolar bone and periodontal tissues in systemic periodontoclasia are identical with those found in infantile scurvy.

The discovery of follicular hyperkeratosis in 84 per cent. of the men in this series is interesting. PRAMZANTOV (1940) considered this condition to be due to dietary deficiency probably of vitamins A, or of fat, and CRAXDOW (1940) developed the condition while on a diet containing no vitamin C and theoretically adequate quantities of vitamin A.

The desert sore is slow to heal, even after the stage of acute inflammation with suppuration has passed, unlike staphylococcal or other skin infections seen in peacetime. Some of the systemic conditions described by WIMPEL (1940), which produce a delay in wound healing, such as defective nutritional and vitamin balance, might be the cause. A low protein diet and/or a high fat diet prolong the healing period, but neither factor was present in this series. Lack of vitamin C interferes with the formation of collagen fibres and the proliferating mesoderm cells fail to mature. Such a state of affairs might well be one cause of chronicity in the lesions under consideration. This view is probably supported by the results of vitamin C therapy. The appearance of the scar is similar to that of wound in subjects deficient in vitamin C, as described by HUNT (1941). The scars of patients treated with ascorbic acid on the other hand, are normal in appearance and do not break down owing to suppuration beneath. In texture, colour and thickness, they resemble the scar of a wound of the skin of a healthy individual.

If nutritional deficiency is a factor in the causation of desert sores it is probable that a deficiency not of one vitamin alone, but of several, is operative and further work on this subject is required.

*Bacteriol. gr.*—Some microbial species are constantly met with on healthy skin including staphylococci, sarcinae, non-haemolytic streptococci, diphtheroid bacilli, and some aerobic spore-bearing types. The species are generally more numerous on exposed than on protected parts of the body (COLLIER, 1936).

1941) These organisms are of the same groups as those found in desert sores in this series and it is possible that they were merely secondary invaders of the abrasion or wound which was the precursor of the sore

Similarly it is probable that desert sores infected with the diphtheria bacillus have been secondarily invaded "Cutaneous diphtheria is not a primary clinical entity but a superimposed infection on an existing lesion" (*Army Medical Directorate Bulletin*, 1942) Furthermore, cutaneous diphtheria occurs when faucial diphtheria or carriers are present In the Sinai desert, when diphtheritic desert sores were common, there was an extensive outbreak of faucial diphtheria and there were many unsuspected carriers (*History of the Great War*, 1924) When wound diphtheria became endemic in Breslau in 1919-20 the outbreak was synchronous with an enormous increase of pharyngeal diphtheria (MELCHIOR, 1940) A number of cases of pharyngeal and cutaneous diphtheria occurred among British troops in Northern Persia early in 1943, and an outbreak of faucial and cutaneous diphtheria in Northern Palestine in September to December, 1940, was reported by CAMERON and MUIR (1942, 1943)

While it is agreed by most observers that cutaneous diphtheritic sores only occur when faucial diphtheria is present and in parallel outbreaks, yet it does not seem to be appreciated that cutaneous diphtheritic sores do not occur during every outbreak of faucial diphtheria, but probably only under conditions when desert sore or some other chronic skin lesions are present Thus in the Sinai desert, in the Northern Persian and in the Northern Palestine outbreaks, desert sores were present beforehand, and it is probable that the outbreaks of cutaneous diphtheria were due to the secondary infection of these Cutaneous diphtheria was rare in Iraq during the period of investigation of this series of desert sores, although the latter were common, because faucial diphtheria was only occasionally seen (Eight cases were admitted to the hospital between 1st January and 1st November 1942)

### CONCLUSION

Assessing the evidence as a whole it would appear not unreasonable to suppose that desert sores are the result of trauma affecting skin whose resistance to infection is lowered, the resulting break in continuity becoming secondarily infected by organisms usually present in the skin or by diphtheria bacilli if carriers are near The lowered resistance to infection and the slow rate of healing may be due to dietary deficiency, and some evidence, though inconclusive, is produced here that this is so

### SUMMARY

The available literature regarding desert sores is reviewed and a series of sixty three cases seen in Iraq is considered with a view to determining the

ætiology of the condition. The results of treatment with vitamin supplements are described and the ætiology is discussed.

It is tentatively suggested that the following are the important factors in the ætiology of desert sore —

- 1 Trauma of the skin.
2. Lowered resistance of the skin to infection, and delayed wound healing probably due to nutritional deficiency
- 3 Infection of the skin at the site of injury and later at other places by organisms locally resident or conveyed by carrier

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# HETEROPHILE ANTIBODIES IN TRYPANOSOMIASIS

BY  
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The type of reaction that occurs between an antigen and an antibody unrelated in their origin is generally described as heterophile, or heterogeneous

A heterophile antigen, as defined by FORSSMAN (1911) is one which when injected into certain animals, evokes the production of antibodies which can be demonstrated by their reaction with antigens other than those involved in their production. The particular antigen that is generally known as Forssman's antigen gives rise in rabbits to the production of haemolysins and agglutinins against sheep cells and is widely distributed in animal cells and tissues.

The presence of heterophile antibodies in man has been demonstrated following injections of horse serum (DAVIDSOHN, 1929), and in the serum of cases of infective mononucleosis (PAUL and BUNNELL, 1932). In the latter case the demonstration of heterophile antibodies and their selective absorption by beef cells is of diagnostic significance.

Although FORSSMAN's antibody is at present the most familiar heterophile antibody with a significance in clinical medicine, a variety of these antibodies have already been described.

\* I wish to express my indebtedness to Colonel R. G. GORDON, late R.A.M.C., for his encouragement during the early stages of this work, to Dr M. VAN DEN ENDE for his valuable assistance in its preparation for publication, and to Major-General L. T. POOLE for his permission to publish this paper.

Heterophile sheep cell agglutinins have been demonstrated —

(1) *In normal serum* where they are usually present in very low titre so that following their absorption by varying antigens it is often exceedingly difficult to demonstrate the presence of residual agglutinin. Investigation of the high titred sera, however has shown that the agglutinins can be absorbed by both sheep cells and fresh or boiled guineapig kidney both of which are Forssman's antigen carriers. They are on the other hand, poorly absorbed by boiled beef cells.

(2) *Following injections of horse serum* when the agglutinins evolved are absorbed by a wide variety of antigens, including the cells of horse sheep goat, ox, rabbit pig dog and guineapig. These include a number of non-Forssman antigens.

(3) *In patients suffering from infective mononucleosis* when the agglutinins present are strongly absorbed by beef cells (non-Forssman) and by the cells of sheep, goat and horse, but are poorly absorbed by guineapig kidney.

#### MATERIALS AND METHODS.

There is considerable disagreement in the literature concerning the sheep cell agglutinin content of normal serum (STUART *et al.* 1934 and BARRETT 1941). The varying results obtained appear to depend to a large extent on a variety of factors, such as the temperature at which the test is performed, the concentration of sheep cells employed, whether the titre is expressed in terms of final or serum dilutions, the method of standardizing sheep cell suspensions, and differences in the methods employed for reading the tests, etc.

The purpose of the present paper is to describe the testing of heterophile agglutinins in relatively high titres in cases of trypanosomiasis in British West Africa, and of attempts to investigate the nature of the antibodies with a view to determining their possible relationship with those that occur in normal serum, and in the serum of patients suffering from infective mononucleosis, and serum sickness.

In view of these wide variations in normal agglutinins, previously reported, it was decided in the present work to determine the local range of normal variations, in order better to assess the value of the finding of a heterophile response in cases of trypanosomiasis and to employ and fully describe the technique most commonly adopted in the routine performance of the Paul-Bunnell test.

Over a period of 18 months 100 sera from confirmed cases of trypanosomiasis were examined. Sera were taken at all stages of the disease but unfortunately in many cases few or no clinical data were available other than the mode of diagnosis. In over 90 per cent. of the cases examined *Trypanosoma gambiense* was present in the enlarged cervical glands. One hundred control sera from patients suffering from a wide range of tropical and non-tropical

diseases, as well as from a large number of normal individuals, were also examined

## TECHNIQUES

### SHEEP CELL AGGLUTININ ESTIMATION

The method used was largely that described by DAVIDSOHN (1938). The sheep's blood was defibrinated and stored at 5° C. The blood was kept for at least 24 hours before use, and was discarded 5 days after it had been withdrawn. Before each test the cells were washed in three changes of normal saline, and a 2 per cent suspension in normal saline was prepared from the spun deposit after the last washing.

Sera to be titrated were inactivated by heating for 20 minutes at 56° C.

0.25 c.c. of serum dilutions ranging from 1/5 to 1/5,000 were set up in small tubes with an internal diameter of about 9 mm. To each of these 0.1 c.c. of the 2 per cent cell suspension was added. Final serum dilutions will range from 1/7 to 1/7,168. The tubes were then incubated for 1 hour at 37° C., and subsequently placed in the refrigerator overnight. Before reading on the following morning, the tubes were re-incubated for a further hour at 37° C. On removal from the incubator the tubes were gently tapped with the forefinger, and inverted to resuspend the cells. The tubes were read by naked eye in a good light, but if there was any doubt as to the end-point, the tube in question was examined with the aid of the concave surface of a microscope mirror, along with the control tube. Previous use of this technique had shown a titre of 1/200 or over to be highly suggestive of a diagnosis of infective mononucleosis.

### ABSORPTION TEST

Guinea-pig kidneys were kept frozen until required. After stripping off the capsule and perirenal fat, they were cut up into small pieces. These were then washed in saline until the washings were blood-free. The tissue was then mashed to a fine pulp using as much saline as was necessary. The suspension was then heated in a boiling water bath for 1 hour. When cool it was further emulsified in saline, centrifuged, and a 20 per cent suspension of the deposit prepared in 0.5 per cent phenol saline. The emulsion thus prepared was kept in the refrigerator, and was considered suitable for use for some months.

Fresh ox cells were washed in several changes of saline until the supernatant fluid was clear. They were then suspended in four times the volume of saline as that of the packed cells. This suspension was then heated in a boiling water-bath for 1 hour, and the fluid lost was made up with further saline. Phenol was added to 0.5 per cent. This suspension can also be kept for some months in the refrigerator.

The test was carried out by allowing a mixture of 1 c.c. of antigen and



0.2 c.c. of inactivated serum to stand for 1 hour at room temperature, shaking every 15 minutes. The mixture was then centrifuged at 1,500 r.p.m. for 10 minutes, and the supernatant fluid was pipetted off. A row of 8 tubes was now prepared, and 0.25 c.c. of saline was added to Tubes 2 to 8. 0.25 c.c. of absorbed serum was added to Tubes 1 and 2, and doubling dilutions prepared from Tubes 2 to 8, discarding 0.25 c.c. from the last tube. 0.1 c.c. of the 2 per cent. suspension of sheep cells was added to all tubes, which were then shaken well. Serum dilutions ranged from 1/7 to 1/896. The tubes were then incubated as described above.

### RESULTS.

Table I shows the mode of diagnosis in the 100 confirmed cases of trypanosomiasis, and indicates the distribution of pyrexial cases in both groups.

Table II shows the frequency distribution of the sheep cell agglutinin titres in the positive and control groups.

Table III shows the rise in titre noted in three early cases actually under observation, along with four other later stage cases where serial agglutinations were also carried out.

Table IV and V show the titres following absorption with guinea pig kidney and beef cells of a small number of sera with high agglutinin titres from the control group, and twenty sera from the trypanosomiasis group.

Tables VI and VII show the agglutination titres of 50 Kahn positive and negative sera in control and trypanosomiasis groups.

Autohaemagglutinins such as were reported by KANTLACK, DURHAM and BLANFORD (1898) to occur in the blood of humans and animals infected with trypanosomiasis though constantly looked for were found in only four cases and in none of these at a titre of more than 1/8. The blood groups of these

TABLE I.  
POSITIVE SERA (100).

<i>T. gambiense</i> present in	Number of cases
Blood only	3 (3 pyrexial)
Gland juice only	66 (1 pyrexial)
C.S.F. only	1
Blood, and gland juice	11 (8 pyrexial)
Gland juice only accompanied by changes in C.S.F.	18

CONTROL SERA (100)

All these cases had negative blood films for *T. gambiense* and showed no clinical features of trypanosomiasis. Fourteen of the cases included in this group were pyrexial.

cases were unfortunately not ascertained. The phenomenon of autoagglutination was also observed in a small number of cases, which, although showing no evidence of infection with *T. gambiense* had enlarged spleens probably due to chronic malarial infection.

TABLE II  
FREQUENCY DISTRIBUTION OF AGGLUTINATION TITRES

	1 7	1 14	1 28	1 56	1 112	1 224	1 448	1 896	1 1,792
Positive sera	3	6	18	28	19	17	6	2	1
Control sera	12	30	38	16	4	—	—	—	—

TABLE III

Case No	<i>T. gambiense</i> present in		Pyrexia	Serial agglutinations			
	Blood	Gland		1	2	3	4
I	+	+	+	1 112 (10)	1 224 (15)	1 224 (21)	1 224 (30)
XXVII	+	+	+	1 112 (2)	1 448 (12)	1 448 (16)	
XLI	—	+	—	1 56 (3)	1 56 (10)	1 224 (16)	
LXVII	—	+	—	1 112 (4)	1 112 (16)		
LXX	—	+	—	1 112 (3)	1 112 (10)	1 112 (30)	
LXXX	—	+	—	1 224 (1)	1 224 (30)		
XC	—	+	—	1 224 (2)	1 224 (16)	1 112 (42)	

Figures in brackets indicate days after admission to hospital

TABLE IV  
CONTROL SERA

Case No	Sheep cell agglutinin titre	Titre after absorption with	
		Beef cells	Guinea pig kidney
XXV	1 56	1 28 (wk.)	<1 7
XXXV	1 28	1 28	<1 7
XLIII	1 28	1 14	<1 7
XLVII	1 112	1 56	<1 7
XLIX	1 28	1 28	<1 7

TABLE V  
POSITIVE SERA.

Case No.	Sheep cell agglutination titre	Titre after absorption with	
		Beef cells.	Onchocerca lucorum
XXXVIII	1 448	1 84	1 84
XLI	1 224	1 7	1 14
XLII	1 112	1 14	1 56
XLIII	1 84	1 14	1 14
XLIV	1 112	1 7	1 8
LXIV	1 84	1 14	1 14
LXVII	1 112	1 14	1 28
LXXIX	1 224	1 28	1 28
LXX	1 11	1 28	1 28
LXXXVII	1 56	1 14	<1 7
LXXXIX	1 112	1 14	1 28
LXXX	1 224	1 28	1 28
LXXXIV	1 28	<1 7	<1 7
LXXXVI	1 11	1 28	1 28
LXXXVII	1 56	1 14	1 14
XC	1 224	1 84	1 112 (k)
XCV	1 448	1 84	1 56
XCVI	1 28	<1 7	<1 7
XCVII	1 112	1 8	<1 7
XCVIII	1 848	1 112	1 224
Average percentage absorption		84.5%	80.0%

TABLE VI  
CONTROL SERA (50).

	Frequency distribution of agglutination titres						
	1 7	1 14	1 28	1 56	1 112	1 224	1 448
kahn positive (16)	4	6	5	1	—	—	—
kahn negative (34)	4	8	12	6	—	—	—

TABLE VII  
POSITIVE SERA (50)

	Frequency distribution of agglutination titres							
	1 7	1 14	1 28	1 56	1 112	1 224	1 448	1 896
Kahn positive (15)	—	—	1	4	6	2	2	—
Kahn negative (35)	—	1	4	11	7	9	2	1

### DISCUSSION

The foregoing survey of 100 sera from confirmed cases of trypanosomiasis suggests that the finding of sheep cell agglutinins in this disease is no chance one, and that an agglutinin titre significantly higher than the normal level may be expected in at least 30 per cent of cases—50 per cent of the cases in this series if the four border-line cases in the control group are disregarded. It is possible that although these four cases showed no clinical or laboratory evidence of trypanosomiasis, they were, in fact latent stages of the disease.

The rise in titre of three of the cases in this series, while suggestive of a specific reaction, is not an altogether convincing finding, as although no similarly high titres were noted among the pyrexial controls the possibility of a non specific stimulation of antibodies cannot be excluded.

The absorption tests, however, provide some evidence to counter this possibility and suggest that the antibodies that have been demonstrated in the serum of patients suffering from trypanosomiasis are in certain respects unique. The absorption of the agglutinins in cases of trypanosomiasis by beef corpuscles which do not contain Forssman's antigen, differentiate them from those present in normal serum. The antibody present in cases of infective mononucleosis differs in that it is not absorbed by guineapig kidney, while the syphilis antibody cannot be associated with that present in trypanosomiasis, as the latter occurs equally in Kahn positive and negative sera.

The differentiation of the trypanosomiasis agglutinins from those present in serum sickness is, however, less clear, as in both cases there is a considerable degree of absorption with both the antigens employed.

A possible relationship of the antigen concerned in trypanosomiasis with that common to Group A human cells and sheep cells requires further investigation.

The mechanism of the production of the antibodies in trypanosomiasis is obscure, and the responsible antigen may be either intrinsic or extrinsic in origin.

In the former case it may be that the antibody is evoked by an antigen

resulting from the breakdown of a body protein. That such an occurrence is possible has been shown by the experimental production of homologous organ-specific antibodies (SCHWENTKER and CONFLOER, 1939; SCHWENTKER and RIVERS 1934 and SAHADEL, 1936). In a recent paper on heterogenetic antibodies in acute hepatitis, EATON MURPHY and HANFORD (1944) speculated on the possibility of an *in vitro* autogenous antigen-antibody reaction. In trypanosomiasis similar manifestations of hypersensitivity to those seen in serum sickness, and reported by the above workers in acute hepatitis are not infrequently seen in the early stages of the disease, and it may be that here, too, such a reaction is responsible.

If, however the provoking antigen be in fact intrinsic, it should be possible to demonstrate at some phase of the disease the presence, in the serum of the patient of an antigen which will react with an antibody in serum obtained from a case in a later stage of the disease, or in convalescent serum. Such a reaction would be analogous to that shown to occur in cases of acute hepatitis, following yellow fever inoculation with material containing heterogenic human serum.

An alternative, and more probable hypothesis is that the phenomenon is a heterologous reaction, and that we are, in fact measuring, both in the case of infective mononucleosis and trypanosomiasis, a fraction of the antibodies evoked by the corresponding disease-producing agent. In both diseases the aetiological agent has an antigenic fraction in common with the sheep cell.

It is obvious that further work must be done on this subject to demonstrate more conclusively a specific rise in antibody titre during the course of the disease. The antibody must be measured quantitatively at different stages of the disease, and attempts be made to correlate it with other immunological responses. Some of this work could readily be done with experimental animal using, for example antitrypanosome rat sera, both for the demonstration of a specific rise in agglutinin titre and for the absorption tests with pathogenic trypanosome suspensions.

There does not appear to be any demonstrable relationship between the presence of sheep cell agglutinins to high titre and a positive Kahn reaction.

The chief significance of the demonstration of sheep cell agglutinins in the serum of a considerable percentage of cases of trypanosomiasis undoubtedly lies in the fact that in the tropics where trypanosomiasis occurs or in patients recently returned from such areas, a diagnostic titre for sheep cell agglutinins, associated with the particular disease syndrome of glandular enlargement (especially of the cervical group) and an atypical blood picture, with or without pyrexia, can no longer be regarded as being sufficiently suggestive of infective mononucleosis to preclude further and repeated investigation. I have been assured that, prior to 1943 in one West African General Hospital alone more than one European case had been diagnosed on the above basis.

as infective mononucleosis, and discharged from hospital shortly following the subsidence of pyrexia without further investigation

It seems possible from the results of the absorption tests in this series, that some differentiation may be made between the two disease conditions in the relatively early stages by the absorption of the sheep cell agglutinins with guineapig kidney

### SUMMARY

1 One hundred sera from cases of trypanosomiasis taken at varying stages of the disease have shown that the sheep cell agglutinin titre is significantly higher than in a series of one hundred control sera taken from patients suffering from a wide variety of tropical diseases, and from normal individuals

2 The antibody present in these cases of trypanosomiasis is strongly but not completely absorbed by both beef cells and guineapig kidney

3 The differences between this and other heterogenetic antibodies have been noted

4 Possible sources of the antigen responsible for eliciting this heterophile antibody response have been considered, and further lines of investigation are suggested

5 Care must be taken before a diagnosis of infective mononucleosis is made in the tropics, or in patients recently returned from the tropics

6 The Kahn reaction does not appear to influence or be influenced by the occurrence, to high titre of sheep cell agglutinins in trypanosomiasis

7 Autohaemagglutination was not noted as a constant feature in trypanosomiasis

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# TSETSE FLY REPLELLMENTS

BY

G M FINDLAY,\*

J HARDWICKE

AND

A J PHELPS

Substances capable of deterring the tsetse fly from biting have received little attention. HORNBY and FRANCH (1943) found that pyrethrum protected cattle and donkeys, while HOLDEN and FINDLAY (1944) showed that pyrethrum in a vanishing cream base had a repellent action when applied to the human skin.

A number of other compounds have now been tested for repellent action, the technique being similar to that used by HOLDEN and FINDLAY (1944). The tsetse were almost exclusively *Glossina palpalis*. All the compounds were in solution.

## EXPERIMENTAL RESULTS

The compounds used and the results obtained are shown in Table I. The figures recorded are the totals obtained from at least two experiments made on different days. In the case of Formula 622, six separate experiments were made.

\* Our thanks are due to Colonel LESLIE E. KNAPP, U.S.A.A.F., for a supply of the compounds tested.



It will be seen that *N*- $\alpha$ -amylsuccinimide, 2 phenyl-cyclohexanol and *n*-butyl-*dl* malate all reduced the number of tsetse which settled and bit. The most striking results were obtained with indalone and Formula 622, while Rutger's 612 had no significant action.

TABLE 1.  
Tsetse FLY REPELLENTS

Compound	Controls		Treated	
	Number of tsetse		Number of tsetse	
	Settling	Biting	Settling	Biting
<i>N</i> - $\alpha$ -Butyrylphthalimide	39	27	41	23
2-Phenylethyl- $\alpha$ -hydroxyisobutyrate	39	27	25	23
<i>N</i> - $\alpha$ -Amylsuccinimide	147	119	88	23
Benzyl ether	82	67	79	64
2 Phenyl-cyclohexanol	77	68	42	21
<i>n</i> -Butyl- <i>dl</i> malate	74	65	43	29
Ethylbenzene-diol-1, 2 (Rutger's 612)	94	39	67	46
-, 2' Dimethyl-2-carboxyoxycarbonyl- 7-pyrone (Indalone)	68	32	21	11
Formula 622 (Dimethyl phthalate 6 parts, indalone 2 parts, Rutger's 612 2 parts)	217	162	74	23

These compounds under the trade mark Slast, are manufactured by the Gallenkamp Chemical Corporation, Windsor, Vermont, for the Slast Co. Inc., New York, N.Y.

#### *Duration of Effectiveness of Indalone and Formula 622*

To determine the time during which these compounds were effective they were applied at intervals of from 1 to 8 hours before exposure to tsetse bites. The results were as follows —

Interval between application and exposure.	Controls		Indalone		622	
	Number of tsetse		Number of tsetse		Number of tsetse	
	Settling	Biting	Settling	Biting	Settling	Biting
1 hour	67	58	12	5	16	6
2 hours	82	46	14	4	14	6
4 hours	60	31	22	6	26	7
6 hours	32	30	26	18	22	27
8 hours	48	33	24	22	26	20

Both compounds thus showed a loss of efficiency between 4 and 6 hours after application

*The Effect of Sunlight and Sweating on the Efficiency of Formula 622*

In tests on pyrethrum the question arose whether sweating or exposure to sunlight reduced its efficiency. The conclusion was reached that excessive sweating was the more deleterious. Similar experiments were carried out with Formula 622. Two treated and two control fly-boys sat for 30 minutes exposed to the direct rays of the sun in a tsetse-free area. They then sat for 1 hour just within the shadow of the bush fringing the Volta River. The results were —

Controls		Treated	
Number of tsetse		Number of tsetse	
Settling	Biting	Settling	Biting
52	48	35	30

Some loss of repellent power was noted. To test the effect of heavy sweating, two treated and two untreated fly-boys danced strenuously in the shade for 30 minutes. They were then exposed to tsetse flies as before. The results were —

Controls		Treated	
Number of tsetse		Number of tsetse	
Settling	Biting	Settling	Biting
42	39	19	4

No loss of efficiency was observed

#### DISCUSSION

Of the nine compounds tested for their repellent action on tsetse, Formula 622, indalone, *N*-*n*-amylsuccinimide, 2-phenyl-cyclohexanol, and *n*-butyl-*dl*-malate were all to a certain degree effective

Unfortunately dimethyl phthalate in pure form was not available but Rutger's 61<sup>o</sup> was without action while indalone was active, so that some and possibly all the activity of Formula 622 is due to indalone.

Both Formula 622 and indalone were found to be effective up to 4 hours of application against the bites of West African species of *Culicoides*.

Experiments carried out by Major LEWIS BERNER (1945) with the above nine compounds against anopheline and culicine mosquitoes showed that 2-phenylethyl- $\alpha$  hydroxyisobutyrate was one of the most efficient compounds, while *n*-butyl-*dl* malate and 2-phenyl-cyclohexanol were two of the least efficient. Repellent action against tsetse flies thus does not entirely parallel that against mosquitoes.

### CONCLUSIONS

Under field conditions nine compounds were tested for repellent action against tsetse. Indalone, Formula 622, *N*- $\alpha$  amylsuccinimide, 2-phenyl cyclohexanol and *n*-butyl-*dl* malate possessed some activity.

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GROWTH OF PROTOZOA IN TISSUE CULTURE  
III TRYPANOSOMA CRUZI

BY

FRANK HAWKING,\*

*From the National Institute for Medical Research, London N W 2*

In a previous paper (HAWKING, 1945) a technique has been described by which *Plasmodium gallinaceum* was grown in cultures of chicken macrophages. The technique appeared applicable to other parasites which show an intracellular phase in cells which can be cultivated *in vitro*. The present paper describes the cultivation of *Trypanosoma cruzi* by this means.

TECHNIQUE

The strain of *T. cruzi* was obtained from the Liverpool School of Tropical Medicine through the kindness of Dr E M LOURIE, it was passaged in mice. The tissue cultures were set up by the technique described in the previous paper. The tissue implants were derived from rat embryos about 2 cm long they were attached to the glass by a small drop of fowl plasma. The nutrient medium consisted of rat serum 20 per cent, chick embryo extract 1 to 10 per cent, and Tyrode up to 100 per cent. Phenol red was added as before. The fluid was changed approximately every 5 to 10 days. Tissue implants were taken from the heart, liver or subcutaneous tissue of the rat.

\* Grateful acknowledgements are due to Mr F V WELSH, FRMS, for the photography, and to Miss R J BERSON and Miss V D MARKHAM for technical assistance.

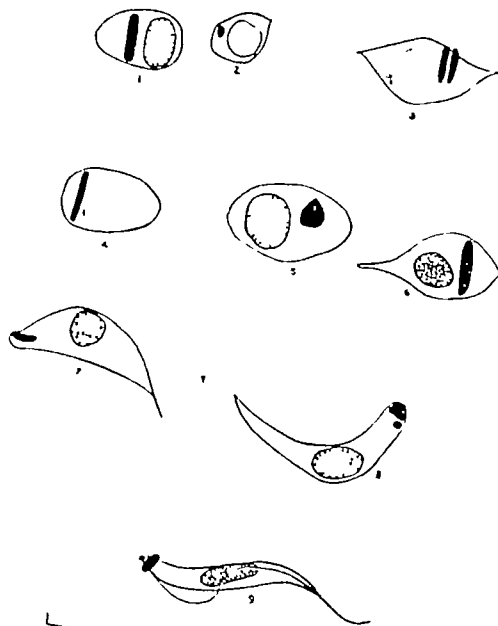
embryo. All grew well implants from the heart continued to pulsate *in vitro* for up to 21 days. After the tissue had been growing for about 3 days a suspension of trypanosomes was obtained from an infected mouse by taking the blood into citrated saline and separating the parasites from the cell by suitable centrifuging. 0.1 to 0.4 c.c. of this suspension was added to each of the flasks which contained about 3 c.c. culture fluid. Growth of the trypanosomes could be followed by inspection under the low power of the microscope for more detailed study cultures were removed from the flask, fixed in Schaudinn's solution without previous washing and stained with Giemsa.

#### EXPERIMENTAL RESULTS.

In the first few days after trypanosomes had been added to the cultures as described above, great numbers of parasites could be seen in the culture fluid. The trypanosomes were extracellular and were as common away from the cells as near to them. Trypanosomes were most numerous on the second day after inoculation but thereafter they became fewer until the 12th day when none were seen by inspection under low magnification. Later a few motile trypanosomes reappeared in the culture fluid and they persisted in varying numbers (usually small) for the rest of the culture. Cultures fixed and stained 2 days after inoculation showed no intracellular forms cultures fixed after 6 or more days showed intracellular forms in increasing numbers. One culture was maintained for 59 days after which it became contaminated by bacteria.

#### MORPHOLOGY OF THE PARASITES.

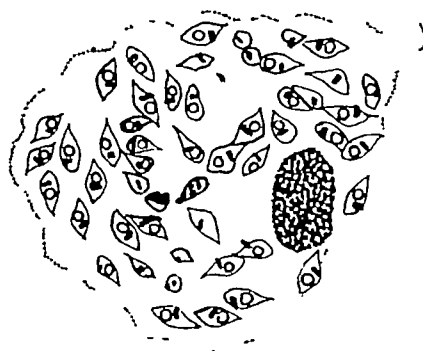
The parasites showed the morphology described by WERYOV (1926) and typical forms are shown in Figs. 1 to 9. The most primitive intracellular form appeared to be the rounded or oval body with a prominent rod shaped parabasal body staining dark red and a round or oval nucleus staining less deeply. Developing from this there were all intermediate stages with various degrees of elongation until the complete trypanosome was formed and became extracellular. The parabasal body was always prominent and stained deeply usually it was rod-shaped and straight sometimes it was curved, sometimes it was globular. In some parasites it was duplicated as shown in Fig. 3. In the mature trypanosomes and in the almost mature intracellular forms, a tiny granule could be seen close to the parabasal body (? blepharoplast) Figs. 8 and 9. The nucleus sometimes stained fairly darkly sometimes it was pale and inconspicuous, sometimes it appeared as an empty space with a small central nucleolus; forms containing two nuclei were rare. The flagellum could rarely be distinguished. The numbers of parasites in the cells varied the early forms might be few or many the more mature forms seemed very numerous distending the cell which generally appeared to be degenerating. The parasites lay in the cytoplasm of the cell and there was a cyst wall



Figs 1-8—Various stages in the development of the intracellular forms of *T. cruzi*  
 FIG 9—An extracellular trypomastigote  
 Figs 1-9 were drawn directly from the preparations by FH Magnification as shown  
 by the scale



10



11

FIG 10—Leishmanoid forms in cardiac muscle cell, 4 days after the addition of trypanosomes  $\times 1250$   
 FIG 11—Forms beginning to elongate, in macrophage from embryonic liver, 10 days after the addition of trypanosomes  $\times 1250$   
 Figs 10 and 11 drawn by V D M from photographs

around them. In some cultures a few leishmanoid forms were present extracellularly; trauma during fixation is negligible in this technique. In one heavily infected culture, a few cells were seen in which the nuclei and outlines of the parasites were practically invisible and the chief evidence for their presence consisted of a number of tiny parabasal bodies spaced at appropriate intervals throughout the cytoplasm of the host cell, the explanation of these appearances, whether due to degeneration, was not clear.

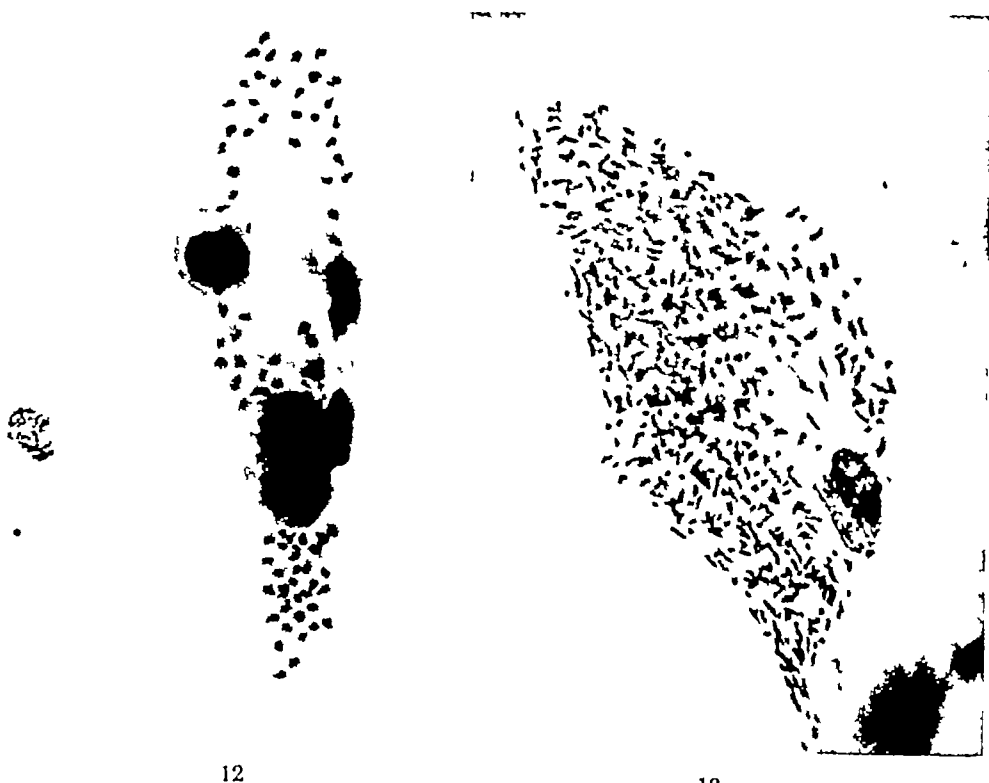
Infection tended to be concentrated in a few cells which were swollen with parasites, while other cells were free. Parasites occurred in cardiac muscle cells and in macrophages. They also occurred in elongated cells with processes at each end, which somewhat resembled fibroblasts, but were probably reticular-endothelial cells. In many cases the cell appeared to be damaged by the presence of the parasites, the cytoplasm becoming swollen, vacuolated and empty in appearance the nucleus becoming dark, pyknotic, and sometimes blurred.

MEXER (1942) has reported that *T. cruzi* could be grown in cultures of brain of chick embryo. His experiment was repeated and confirmed. A suspension of trypanosomes prepared from mouse blood was added to a culture of brain of chick embryo grown by the technique described above. The fluid medium consisted of 20 per cent. fowl serum in Tyrode as above. After some days a fair growth of leishmanoid forms was found inside the cells.

#### DISCUSSION.

Growth of *T. cruzi* in tissue culture has been described by KORON *et al.* (1935). They grew tissue from rat embryos in plasma-embryo extract medium in slide cultures after 24 hours tritidial forms of the parasite derived from cultures in semisolid blood agar medium were added. Growth of the parasites inside macrophages and heart muscle could be seen and trypanosomes of the type found in blood appeared after 5 days. Cultures were maintained for 7 days. In a later paper KORON *et al.* (1937) describe the application of this technique for examination of the effect of arsenical compounds upon these parasites. MEXER (1942) describes experiments in which slide cultures were made of chick embryo brain, after 24 hours a drop of a culture of *T. cruzi* was added, the parasites underwent the usual cycle of development in the cells of the culture. He refers to an earlier paper of ROMANA and MEXER in the same year in which they had shown that growth occurred in cultures in fibroblasts, histiocytes epithelial, muscle and nerve cells.

The present experiments were undertaken mainly to explore the wider application of the technique developed for the growth of *P. gallinaceum* and no attempt has been made to study the morphology or growth requirements of the trypanosome in detail. Enough has been done to show that cultures of *T. cru* are easy to obtain and to maintain by this technique.



12

13

FIG 12 —Early leishmanoid forms in an elongated (? reticulo-endothelial) cell from skin, 14 days after the addition of trypanosomes Three small cells are applied to the outside of this cell  $\times 1000$

FIG 13 —Almost mature intracellular forms in a cardiac muscle cell 23 days after the addition of trypanosomes  $\times 1000$



around them. In some cultures a few leishmanoid forms were present extracellularly; trauma during fixation is negligible in this technique. In one heavily infected culture a few cells were seen in which the nuclei and outlines of the parasites were practically invisible and the chief evidence for their presence consisted of a number of tiny parabasal bodies spaced at appropriate intervals throughout the cytoplasm of the host cell; the explanation of these appearances, whether due to degeneration was not clear.

Infection tended to be concentrated in a few cells which were swollen with parasites, while other cells were free. Parasites occurred in cardiac muscle cells and in macrophages. They also occurred in elongated cells with processes at each end, which somewhat resembled fibroblasts, but were probably reticulo-endothelial cells. In many cases the cell appeared to be damaged by the presence of the parasites, the cytoplasm becoming swollen, vacuolated and empty in appearance, the nucleus becoming dark, pyknotic, and sometimes blurred.

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The present experiment were undertaken mainly to explore the wider application of the technique developed for the growth of *P. gallinaceum* and no attempt has been made to study the morphology or growth requirement of the trypanosome in detail. Enough has been done to show that cultures of *T. cruzi* are easy to obtain and to maintain by this technique.

Such cultures offer favourable conditions for study of the intracellular stages of *T. cruzi* and of their reactions to drugs

#### SUMMARY

The technique used for cultivation of exoerythrocytic forms of *P. gallinaceum* has been applied to the growth of *Trypanosoma cruzi* also. A suspension of trypanosomes from the blood of an infected mouse was added to cultures of rat embryo. During the first 2 days great numbers of trypanosomes were present in the culture fluid, not particularly associated with the cells. Then they gradually became fewer until by about the 12th day they were rare or absent. Later small numbers reappeared in the culture fluid and persisted there until the end of the culture. Stained preparations made after 6 days or more showed great numbers of intracellular parasites in all stages between the rounded leishmanoid form and the mature trypanosome form. The parasite occurred in cardiac muscle fibres, in macrophages and in elongated cells with processes (probably reticulo-endothelial cells). Cultures have been maintained for 59 days. As described by MEYER (1942) *T. cruzi* could also be grown in cultures from the brain of chick embryo.

This technique offers favourable conditions for study of the intracellular development of *T. cruzi* and of the action of drugs upon it.

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## CORRESPONDENCE.

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### KAPOSI'S MULTIPLE IDIOPATHIC HAEMORRHAGIC SARCOMA

*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

With reference to the communication by DENNISON and WINSTON EVANS (*Trans R Soc trop Med Hyg*, 1946, 39, 521) recording a case of Kaposi's sarcoma in a West African soldier, it should be noted that, taking into account the relatively unobserved character of diseases in West Africa, this condition cannot be regarded as particularly rare SMITH and ELMES (1934)\* in classifying 500 malignant tumours received by them at the Medical Research Institute, Yaba, Nigeria, found that ten were Kaposi's sarcoma During the war five cases of Kaposi's sarcoma were treated among West African soldiers in Army Hospitals in West Africa Including the case reported by DENNISON and WINSTON EVANS, four were in Nigerians and one in a Twi-speaking native of the Gold Coast Since the total African Army population was approximately a quarter of a million, this gives an incidence of at least one in 50,000 among the young adult male population of West Africa The common occurrence of the primary lesions around the ankles and on the dorsum of the foot suggests that trauma may play some part in setting up the condition, more especially as no case was seen in literate and booted African clerks Secondary nodules were present on the skin of the arms and thighs in two cases

In conjunction with Dr B G T ELMES, attempts were made in two instances to transmit the disease to experimental animals including one chimpanzee, baboons *cercopithecus* and Patas monkeys, rabbits, guineapigs and mice The chimpanzee was inoculated intradermally with tumour material

\* SMITH, E C & ELMES, B G T (1934) *Ann trop Med Parasit*, 28, 461



Rather less than a year later he returned with the other foot in exactly the same condition. I cannot remember clearly the distribution of the nodules at this time, but they were markedly fewer than when he had been discharged. The foot was swollen and ulcerated and very painful, and the patient was asking for a second amputation. This was carried out, and again he made an excellent recovery. That was 2 years ago, and I have seen him frequently since. He walks up to the hospital with a beaming face on two wooden legs. All traces of the nodules have disappeared.

I may perhaps add to DENNISON and EVANS' histological and diagnostic notes a word about the course of this unusual condition. EWING (1940, *Neoplastic Diseases*, 4th ed. p. 279-282) says that Kaposi's disease, while it may not be a truly malignant neoplasm, 'is often complicated by infections producing refractory painful and suppurating processes which deplete the patient'. The course is slowly progressive, running from 1 to 25 years, but averaging 5 to 10 years. Remissions lasting for many years may occur, and spontaneous cures have been reported. Early treatment with radiation and arsenic is recommended. It would appear therefore that the ultimate prognosis of the present case is quite uncertain.

I am, etc.,

R B LEECH

Kampala, Uganda

## A SIMPLE DEVICE FOR THE APPLICATION OF DDT LARVICIDE

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

By a modification of an ordinary flit sprayer it is possible to facilitate spraying of DDT larvicide in treating anopheles breeding places, mainly of *superpictus* and *bifurcatus*.

The "apparatus" is an ordinary flit gun, the tubing and the piston handle extended to a length of about 3 to 3½ feet. The additional tubing and piston handle can be made by any local tinsmith.

The usefulness of this extension is that a person can treat a breeding place without having to stoop, and breeding places covered with weeds and grass, or edges which are of soft mud, can be reached and treated with safety.

The droplets from this type of larvicide gun should be slightly larger than those discharged by the ordinary insecticide "Flit" sprayers. This can be done by having the diameter of the tubing which leads from the container slightly enlarged.

suspended in saline, the monkeys, rabbits and guinea-pigs intradermally subcutaneously and intratesticularly and the mice intracerebrally. No evidence of infection occurred in an observation period of 3 months. When tumour material was injected into the anterior chamber of the guinea-pig's eye some growth of cells was observed and the condition could be transmitted in series for three passages. In two instances patients suffering from Kaposi's sarcoma were inoculated intradermally in the leg with tumour material ground up in physiological saline. Small nodules developed at the sites of inoculation in from 3 to 4 weeks. The same material passed through a Seitz filter and similarly inoculated did not produce lesions during an observation period of 2 months.

While the possibility of a virus aetiology is by no means excluded, in view of the long incubation period of such conditions as human warts and molluscum contagiosum, it is obvious that Kaposi's sarcoma is not readily transmissible to laboratory animals.

I am, etc.,

G. M. FINDLAY

*late Consulting Physician on Tropical Medicine  
West African Command*

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*To the Editor TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

In view of the report of a case of multiple idiopathic haemorrhagic sarcoma occurring in a West African (DEMONSON W and EVANS W (1946), *Trans. R. Soc. Trop. Med. Hyg.* 39: 521) it may be of interest to record a case in an East African. As I am at present in England I cannot refer to the notes, but I well remember the main features of the case, the course of which also was of some interest.

The patient was a male Ugandan, aged about 30 years. He was admitted to Hospital, I think in 1943 also with symptoms resembling Madura foot but with numerous nodules on the foot and leg. A biopsy was performed and the pathologist reported multiple idiopathic haemorrhagic sarcoma of Kaposi. The nodules increased rapidly in number and appeared on other parts of the body notably the arm. The patient was far from well and appeared to be going downhill. The nodules were painless but the foot was extremely painful and no local or general treatment was of any avail. In spite of the uncertain course of the disease, amputation seemed justified simply to relieve the pain, and the patient willingly agreed. The operation was performed above the knee, and he made a remarkably good recovery. His general health improved, and the nodules ceased to increase in number.

Rather less than a year later he returned with the other foot in exactly the same condition. I cannot remember clearly the distribution of the nodules at this time but they were markedly fewer than when he had been discharged. The foot was swollen and ulcerated and very painful, and the patient was asking for a second amputation. This was carried out and again he made an excellent recovery. That was 2 years ago, and I have seen him frequently since. He walks up to the hospital with a beaming face on two wooden legs. All traces of the nodules have disappeared.

I may perhaps add to DENNISON and EVANS' histological and diagnostic notes a word about the course of this unusual condition. LEWIS (1940, *Neoplastic Diseases*, 4th ed. p. 279-282) says that Kaposi's disease, while it may not be a truly malignant neoplasm 'is often complicated by infections producing refractory painful and suppurating processes which deplete the patient'. The course is slowly progressive, running from 1 to 25 years, but averaging 5 to 10 years. Remissions lasting for many years may occur and spontaneous cures have been reported. Early treatment with radiation and arsenic is recommended. It would appear therefore that the ultimate prognosis of the present case is quite uncertain.

I am etc.

R. B. LEECH

Kampala, Uganda

# A SIMPLE DEVICE FOR THE APPLICATION OF DDT LARVICIDE

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

By a modification of an ordinary flit sprayer it is possible to facilitate spraying of DDT larvicide in treating anopheles breeding places, mainly of *superpictus* and *bifurcatus*.

The "apparatus" is an ordinary flit gun, the tubing and the piston handle extended to a length of about 3 to 3½ feet. The additional tubing and piston handle can be made by any local tinsmith.

The usefulness of this extension is that a person can treat a breeding place without having to stoop, and breeding places covered with weeds and grass, or edges which are of soft mud, can be reached and treated with safety.

The droplets from this type of larvicide gun should be slightly larger than those discharged by the ordinary insecticide "Flit" sprayers. This can be done by having the diameter of the tubing which leads from the container slightly enlarged.



This simple type of DDT sprayer has been found to be very useful in Cyprus mainly in treating *superpictus* breeding places and also as a useful means of spraying chicken coops, sheep-folds, stables, caes wells, tree trunks, etc., for the destruction of adult *Anopheles* sheltering therein. The capacity of the container is about one litre. The operator can carry a small supply with him for refill.

The diameter of the tubing should be about  $1\frac{1}{2}$  to 2 inches. This will afford the operator a safe grip on the sprayer. It is advisable that the tin used in the manufacture of this type of sprayer should be non-staining tin. Otherwise the hands of the operator become badly soiled by using inferior material. It is essential that the container be very firmly soldered to the tube to stand the rough work it has to do.

This type of DDT larvicide sprayer was manufactured locally first at 15s. and later at 7s. each. In England it can be manufactured at 4s. to 5s. each. These sprayers have been successfully used in the application of larvicide during last summer and in the present experimental scheme of the *Anopheles* Eradication Service in the Karpas area of this Island.

I have to thank the Acting Director of Medical Services of Cyprus for permission to publish this letter.

I am etc.,

M. Aziz,

*Ch of Health Inspector and Executive Officer  
Anopheles Eradication Service Cyprus.*

Nicosia, Cyprus.

## A CASE OF BLACKWATER FEVER DURING MEPACRINE THERAPY

To the Editor *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

Sir,—

In view of the important paper on the use of certain drugs in the prophylaxis of malaria by N. HAMILTON FAIRLEY (1945 *Trans R Soc Trop Med Hyg* 38: 311), and the widespread use of and faith in prophylactic mepacrine in areas of endemic malaria the following case history may be of interest.

The patient was an Italian Sister of the Roman Catholic Mission at Wau, Equatoria Province, Sudan, an area of hyperendemic malaria where black water fever is well known. Aged 34 years she has been in the area for the last 10 years and has not yet had leave home.

During this long period she had been in the habit of taking 5 gram of quinine two or three times per week, and had had only one attack of malaria, a mild one in 1939. She was due to go home in September this year.

On 17th August she was seen by the Mother Superior, to whom she complained that she had not been feeling very well for the past fortnight, though she had had no specific symptoms. In view of the fact that she was looking pale and thin and was soon due to embark on the long trip down the mosquito-ridden river on her way home the Mother Superior recommended that she should cease to take quinine and that instead she should take a full course of mepacrine three tablets a day for 5 days and then continue to take one tablet a day. This she duly did and by 2nd September she had taken twenty seven tablets.

On this day she felt well until midday when she lunched normally with the other Sisters. In the evening she felt feverish and developed bouts of vomiting and diarrhoea. Her temperature was found to be  $102^{\circ}\text{F}$  and a blood film was taken, but was negative. I was called to see her the following morning after she had passed a fitful night.

On examination her temperature was  $101^{\circ}\text{F}$  and her pulse 108. Her skin was stained yellow and she was observed to have a slight icteric tinge in her conjunctivae. Her tongue was coated her heart and respiratory system normal. Her spleen was enlarged to a finger's breadth below the costal margin, was of a firm consistency and was tender. A second blood film was taken and found to be negative. Her stools were normal in colour.

At 10.0 a.m. she passed urine of a deep black colour, and it was found on examination to be typical of the urine of a case of blackwater fever. An injection of mepacrine 0.3 gramme was given into the buttock, phenobarbitone 2 grains were given by mouth and frequent small draughts of a mixture containing glucose 5 per cent and sod bicarb 2 per cent were ordered. At 1.30 p.m. she passed urine of a lighter colour but still containing haemoglobin and numbers of hyaline and haemoglobin casts. At 3.30 p.m. the specimen was deeper in colour, and at 11.0 p.m. and again at midnight the urine was dark red.

The following day her temperature had dropped to  $100.2^{\circ}\text{F}$ , but she was greatly distressed with pain in the loins and in the bladder, difficulty in breathing and general weakness, and she believed that she was going to die. Mepacrine 0.3 gramme was administered intramuscularly night and morning and phenobarbitone 3 grains during the day. She continued to drink large quantities of the alkaline glucose mixture. At 5.0 a.m. and 7.15 a.m. she passed urine normal in colour, but at 11.45 and again at 1.0 p.m. it became a dark orange. She complained that she had a burning pain on micturition, and a stained film of the deposit from the urine showed numerous streptococci. She passed no more urine that day, and the total for the 24 hours was 770 c.c. A blood count showed 2,200,000 R.B.C.

The following morning her temperature dropped to  $99.4^{\circ}\text{F}$  and she felt very much better. She was given 0.6 gramme mepacrine intramuscularly sulphathiazole 0.5 gramme 4 hourly, and phenobarbitone 1 grain night and morning. By the evening she had passed 1,370 c.c. of normal urine, the pain

in her loins and her bladder had ceased and her breathing had become normal.

Her subsequent progress was uneventful. Her temperature remained around 99.8° F until 9th September when it dropped to normal. She finished her course of sulphathiazole on this day and her urine was clear and free from organisms. She has steadily become stronger ever since.

The interest in this typical case of blackwater fever lies in the fact that the patient had taken a considerable quantity of mepacrine and was still taking it when she developed the illness. The truth of this is vouched for by another Sister and her skin on the first examination showed obvious staining which could not possibly have been due to the mild degree of icterus. In view of this and the sincere character of this Italian Sister there can be little doubt as to the truth of the story.

HAMILTON FAIRLEY (*loc cit*) in his graph of the daily blood concentration of mepacrine on a regime of 0.1 gramme per day showed the peak value to lie between the 25th and 28th days after 2.5 to 2.8 grammes of the drug had been taken, and he states that blackwater should not develop on this regime. This patient had taken 7.7 grammes of mepacrine and it was to have been expected that her blood concentration would be sufficiently high to have given her complete protection.

Dr IAN G. W. HILL (1946, *Trans R Soc trop Med Hyg* 39: 472) mentions one case of blackwater fever in the 14th Army at a time when suppressive mepacrine was vigorously enforced but he does not state whether the patient had definitely taken the regular dose or had defaulted in this respect.

This case must surely be very unusual and it would be of interest and importance to hear of other similar experiences.

I am, Sir, etc.

P. H. ABBOTT

*Medical Inspector Sudan Medical Service*

Wau, Equatoria Province, Sudan.

The previous number of these Transactions Vol 40, No 3,  
was published on December 30th, 1946 ]

TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

VOL 40 No 4 MARCH, 1947

LABORATORY MEETING  
of the Society held at the  
London School of Hygiene and Tropical Medicine,  
Keppel Street, London,  
on  
Thursday, 21st November, 1946, at 8 p m

THE PRESIDENT  
C M WENYON, CMG, CBE, MB, BSC, FR S,  
in the Chair

DEMONSTRATIONS.

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE  
DEPARTMENT OF PARASITOLOGY

Prof H E Shortt and Dr B Malamos

A series of exhibits showing the pre-erythrocytic forms of *Plasmodium gallinaceum* in the brain of the chick at 24-hour intervals following infection by the bites of *Aedes aegypti*. The stages shown commenced from 48 hours and continued up to 7 days

Dr B Malamos

- 1 Lymphogranuloma inguinale virus
  - a In a mouse brain
  - b In cultures of the rabbit cornea
- 2 Very early stages of oriental sore, simulating acne, pustules or boils

DEPARTMENT OF BACTERIOLOGY  
Dr J C Cruickshank and Dr A A Miller

Melioidosis —The bacteriology of *Pfeifferella whittmori* recently isolated from cases  
The clinical features and postmortem findings in a case of melioidosis in

a West African soldier in Rangoon were described. Microphotographs and sections of tissues were shown.

The morphological cultural and biochemical characters of seven strains of *Pfeifferella schottmori* and the lesions produced by experimental inoculation in the guinea pig were demonstrated. These strains had recently been isolated from cases of melioidosis in the Far East by Major Peck and Captain E. J. HARRIS, R.A.M.C.

Dr A. A. MILLER

#### Balantidial dysentery

The clinical features and postmortem findings in a case of balantidial dysentery in an Indian Sepoy were described. Microphotographs and histological sections from the pelvic colon were shown. This case will be published later.

#### DEPARTMENT OF ENTOMOLOGY

Dr Kenneth Mellanby

#### 1 Living material, *Anckimeromyia luteola*.

The Congo floor maggot, the larva of *Anckimeromyia*, lives by sucking blood much like a bed bug. These larvae are resistant to starvation, and can be sent by air from Africa to this country. They feed easily on laboratory animals (rabbit, etc.) Pupae and adults obtained from larvae in the laboratory were exhibited.

#### 2 Adaptations of insects to changes of temperature

The range of distribution of an insect is restricted in several ways one being its reaction to temperature. At the upper end of the scale is the "thermal death point" this seems to be rigidly fixed and is not altered by previous conditioning to different temperatures.

At the lower end of the scale we get the "chill coma temperature" at which cold anaesthesia begins and the still colder temperature at which death ensues. Both these points may often be altered considerably by a process of adaptation. Insects from cultures kept under warm conditions have a substantially higher chill coma temperature than those kept cool. This adaptation occurs rapidly usually within 24 hours, and it is easily reversible so that a heat adapted insect may become "cold adapted" and *vice versa*.

This process may be demonstrated with most insects. The cockroach *Blattella orientalis* was shown here. Some specimens had been kept at 30° C. (heat adapted) others at 15° C. (cold adapted) for 24 hours. When exposed to 5° C. the cold adapted individuals were normally active, the others were immobilized. Both lots were immobilized at 0° C. but on being returned to room temperature (about 20° C.) the cold adapted cockroaches were fully active in under 5 minutes, while the heat adapted ones did not move for about 2 hours.

### 3 Sarcoptic mange in the alpaca, *Lama huanaco* var *paca*

The mite *Sarcoptes scabiei* de Geer attacks many animals including man. At one time it was thought that each species of mammal had its own species of mite, but now it is generally held that there is only one species which can be divided into several biological races.

The South American domestic alpaca (*Lama huanaco* var *paca*) is attacked by *Sarcoptes*, and gives a reaction resembling crusted or Norwegian scabies in man. There is a considerable hypertrophy of the horny layer of the skin, particularly in and around the ears. These crusts were shown to be full of all stages of the mite.

Experiments have been carried out to see whether the mite from the alpaca can infect man. A slide demonstrated showed a biopsy of a burrow produced in a volunteer on whom a fertilized female was placed. A normal burrow was made, and eggs were laid. Other infections gave exactly similar reactions to the human *Sarcoptes*. It does seem, however, that the mites are rather more reluctant than those derived from other humans to burrow, but this point is still in doubt. Morphologically no difference has yet been established between the human and alpacan mites.

### Dr J R Busvine and Mr H C M Parr

#### Laboratory apparatus for testing insecticidal sprays

The machine is designed to apply, for laboratory investigations, residual films of insecticide to pieces of different material up to 1 foot square. Insecticide solution is fed on to the centre of a brass disc spinning at 1,000 to 2,000 r.p.m. which throws off a centrifugal spray of uniform droplets. Materials to be treated are drawn through an arc of this spray.

### Lt-Col J R Audy

#### Epidemiology of scrub typhus in Assam and Burma

The exhibits of charts and photographs represented some of the epidemiological findings of the team based on the Scrub Typhus Research Laboratory, South-East Asia Command, at Imphal. The bacteriological work of this team was undertaken by the G H Q (India) Field Typhus Research Team (Major S LAL KALRA, I A M C). A preliminary report of the team's work is shortly to be published.

The exhibits were grouped as follows —

- (1) Maps and photographs showing the general features of scrub typhus in the Indo-Burma theatre during the war, and emphasizing the high incidence in single units which operated in hyperendemic areas (e.g., over 1,600 cases in a single division in 12 months).
- (2) Biological and epidemiological evidence (apart from the recovery of infection from the mites) that *Trombicula deliensis* is the important vector in the whole theatre.
  - (a) The geographical distribution of *T. deliensis* and *T. akamushi*,

both very closely related alone corresponds to the geographical distribution of scrub typhus. (b) *T. deliensis* larvae have been shown conclusively to have an annual peak incidence during the rains (not shared by any other potential or suspected vector in this theatre) which corresponds closely with the marked seasonal incidence of scrub typhus in areas such as those of Manipur and Lower Burma where there is a definite dry season. (c) Data were presented on which the monthly and annual turnover in numbers of larvae attaching to an average wild rat have been estimated. The turnover of *T. deliensis* greatly exceeds that of any other species encountered, and thus is clearly related to its success as a vector.

(3) The interrelationship between the parasitic larva and its host was illustrated by experimental evidence that both the population and distribution of the mites are related to the population and range of movement of the principal hosts. Of these hosts, the rat\* is considered by far the most generally important for numerical as well as other reasons. The patchy distribution of the disease and also its relationship to terrain are both explicable on this basis. Evidence was presented of an increase of endemicity following local encouragement of rats in neglected camp sites.

(4) Photographs illustrated an example of a hyperendemic focus (a typhus "island") near Imphal. The features of this and many such foci are sufficiently clear and understood to make their anticipation practicable by a study of air photographs.

Dr H S Fuller

#### Illustrations of scrub typhus in Burma and Assam.

A diagram of a portion of the Stilwell Road east of Ledo, Assam, showed the location of certain camp sites between the road and the Tirap River from 11.8 to the 12.4 mile marks. Chinese troops encamped here were involved in an outbreak of scrub typhus during the spring months of 1945. Exceedingly high attack rates occurred in two companies of troops. Their camp areas were separated by a patch of mixed jungle, but there were no geographical barriers between the company areas and the remainder of the areas occupied by their respective battalions. Their only point in common was that the men shared the use of a grassy area at the river's edge for bathing and other purposes. Exposure to attack by trombiculid mites was maximal here, owing to the fact that the men sat or lay in the grass, either naked, or clad only in loose shorts. During the course of the outbreak, *Trombicula deliensis* Walch 1922 was found on boot collections in this small area.

This area at the river's edge was made the central feature of the demonstration. It was referred to as a "hyperinfective pin-point focus, owing to the numbers of cases of scrub typhus acquired here, the presence of a heavy concentration of the vector and the recovery of strains of *Rickettsia orientalis*

Or other locally populous miteid such as *Banficola (Nesbina) bengalensis*.

from mites and rats trapped nearby and in the camp areas proper. Photographs illustrated the river bank in the hyperinfective area, comparing it with the environment along the river in a nearby camp downstream, where hazard of infection was much less.

Photographs of the actual camp sites emphasized the abundance of attractions to rats, and rat harbourages. Photographs taken in June and October showed the amazing degree of regrowth of vegetation in previously cleared areas during 4 months of abandonment, including the monsoon season. On entering such an area, new troops would be forced to clear this vegetation to establish their camp, and, in so doing, they would be heavily exposed to danger of infection with scrub typhus.

These studies were carried out during and after the outbreak by a party of investigators from the China-Burma-India Field Headquarters, United States of America Typhus Commission (1946).

## REFERENCE

MACKIE, THOMAS T, *et al* (1946) *Trans R Soc trop Med Hyg*, 40 (1), pp 15-46

## Dr Rajindar Pal

## Chromosomes of mosquitoes

In several species of mosquitoes biological races or varieties have been discovered which exhibit great biological differences from one another and are more or less genetically distinct as shown by hybridization experiments. These varieties are, however, not readily distinguished in the adult form, but only on certain egg or larval characters. *Anopheles maculipennis* is a typical example.

It is essential as regards malaria transmission to be able to distinguish these varieties as some carry the disease and others do not. In the absence of any morphological characters, it was believed that the salivary gland type of chromosomes of these varieties might be useful in distinguishing different varieties. Moreover, such a study might also throw some light on the controversy whether these varieties should be regarded as such or recognized as independent species.

Though mosquitoes have been the subject of several cytological studies no attention seems to have been paid to differences in the chromosomes of different varieties. SUTTON (1942)\* attempted to obtain polytene chromosomes in *Culex pipiens* and *Aedes aegypti*, but discovered that these chromosomes are very soft and often fragment at "weak spots," thus obscuring the continuity of chromosomes, and may not be suitable for detailed cytological studies.

An attempt was made to obtain polytene chromosomes in *Anopheles maculipennis* var *atroparvus* both in the imagoes and larval stages by feeding the larvae on rich diet of yeast and dog biscuit powder, etc., and freezing the fourth stage larvae for 48 hours in a low temperature cabinet running at 10° C.

Preliminary examination of various tissues such as salivary glands, gut, gonad sheath and malpighian tubules showed that the malpighian tubules give

\* SUTTON, E (1942) *Proc Nat Acad Sci*, 28, 268-272



encountered in the tropics, a white surface emits as much radiant heat of long wavelength as a black surface. It followed from this that no advantage to thermal comfort would be gained by whitewashing interiors and that its value for this purpose depended on its use on external surfaces for reflecting direct solar radiation.

#### DEPARTMENT OF BACTERIOLOGY

Dr J T Duncan

##### *A unique form of Histoplasma.*

The sections were from papulo-carcinate skin lesions on a man aged 63. They showed little aggregations of giant cells in the superficial part of the dermis, in places encroaching on the epidermis, and in the cytoplasm of these cells were numerous large oval thickly capsulated fungal cells measuring 12 to 15  $\mu$  in their longer diameter. There was no inflammatory reaction. In some respects the histological picture suggested an unusual type of blastomycosis (Gilchrist's disease) or of histoplasmosis for the normal parasitic form of *Histoplasma capsulatum* is a small capsulated yeast 3 to 4  $\mu$  in diameter found chiefly in histiocytes. From fresh biopsy material *Histoplasma capsulatum* was isolated vegetating in primary culture at 37° C on blood-sugar in the small yeast form, and at 22° C on various media, in the characteristic mycelial form bearing both the typical large tuberculated chlamydospores and the smaller kind. These two forms were interchangeable in subculture by merely reversing the conditions of cultivation and the mode of transition from the mycelial to the yeast form was illustrated in the demonstration. Occasionally groups of large cells resembling those seen in the lesion were found in some of the earlier cultures of the yeast form, and on a few occasions the entire culture was composed of very large cells suggesting involution forms, but reversion to the small yeast form always occurred in subculture. Experimental animal inoculations, made when the strain had been 3 years under artificial cultivation, gave the following results. Mice injected intravenously with small doses of the living culture in the small yeast form, died after 9 to 12 days and their organs showed the acute lesions of histoplasmosis, in which the histiocytes were filled with small capsulated yeasts 4 to 5  $\mu$  in diameter. However as the infection was very acute and the form used in the inoculation was similar to that seen in the histiocytes, it is not justifiable to conclude on this evidence alone that the small yeast is the normal parasitic form of the strain. Mice inoculated intraperitoneally with the fungus in the small yeast form, and rats similarly inoculated with the mycelial form, developed a chronic infection with no observed effect on the animal health. When the animals were sacrificed, 6 to 8 months later chronic milary lesions were found in the greatly enlarged spleen the liver lungs and other organs and in these lesions the parasite was present in giant cells, in histiocytes or aggregated in nests in the large form indistinguishable from that seen in the patient's skin. This fungus may be a species of *Histoplasma* distinct

from *H capsulatum*, or it may be a variant of this species, possibly approaching *Blastomyces*, but, on the other hand, it may represent merely a less active parasitic form, peculiar to chronic lesions. Although the disease was diagnosed in England, the infection may have been contracted in West Africa, and this may also be significant. The experimental study is proceeding.

### Dr. A. J. Rhodes

The use of the fertile egg in virus and rickettsial research

This demonstration included —

- (1) A series of chick embryos at various stages of development
- (2) Sections and photographs illustrating the development of the respiratory tract of the embryo between the 12th and 16th days
- (3) Preparations illustrating the structure of the normal embryo—air-sac, yolk-sac, chorio-allantois, allantoic cavity and amniotic cavity
- (4) Preparations illustrating the method of inoculating eggs in the yolk-sac, allantoic cavity, amniotic cavity and chorio-allantoic membrane
- (5) "Candling" of eggs

### THE ROSS INSTITUTE

### Dr. R. Ford Tredre

Malaria control measures in the vicinity of the Town and Port of Lagos

In 1942 Lt.-Col. A. B. GILROY, I.M.S., was seconded to West Africa as a Malariaologist, at Lagos his recommendations to the joint Military and Civil Malaria Committee were put into effect under his personal supervision. The permanent control measures consisted in the application of Sir MALCOLM WATSON's method of control of *A. sundaus* in Malaya, namely the exclusion of tidal waters from the anopheline breeding zone by the construction of an embankment with suitably placed tide-gates and the internal drainage of the area so "bunded". GILROY and CHWART have described the application of this method to the swamps in the vicinity of Lagos (*Ann. Trop. Med. & Parasit.*, 1945, 39, 19-40).

At the laboratory meeting a large-scale map of Lagos and vicinity showed the programme for the years 1943-46 of this swamp reclamation method of anopheline control.

The anopheline vectors are *Anopheles gambiae* and *Anopheles melas*. A chart based on BARBER and OLINGER's work (Studies on malaria in Southern Nigeria *Ann. Trop. Med. & Parasit.*, 1931, 25, 461-501) illustrated the average monthly density per room of this mosquito group, then known as *Anopheles costalis*, and the related sporozoite rate. Parasite indices from the same source quoted 61 per cent for all ages and 94 per cent for the 1 to 4 and 5 to 8 years age groups.

The report of the M.O.H. for 1938 was quoted "Population 170,000. Infantile mortality rate 127. Percentage of total deaths registered due to malaria 6.3."

Graphs of comparative monthly densities per room of *A. gambiae* and *A. melas* (1945) at a number of villages in the vicinity of Lagos were shown; these were based on figures provided by Dr R. C. MOUTHEAD THOMSON. The very heavy fall in village anopheline infestation following on adjacent swamp reclamation was illustrated by a graph drawn from a summary of weekly mosquito catches over the period August, 1945 to November 1948 sent by Dr A. GILROY.

The team, Dr A. B. GILROY and Dr L. J. CHWATT are again working together under the D.M.S., Nigeria, and it will be of great interest to follow their annual assessment of anopheline infestation of Lagos and the ultimate effect on malaria incidence resulting from these extensive permanent field control measures.

#### LIVERPOOL SCHOOL OF TROPICAL MEDICINE.

Prof B Macgregor Dr A R D Adams Dr W H H Andrews and Miss M Tottey

- 1 Comparison of clinical effects of single doses of paludrine<sup>o</sup> and mepracrine in the treatment of benign tertian malaria
- 2 Relapse of benign tertian malaria during a course of 100 mg paludrine weekly

#### 1 Treatment of B T relapses with single doses of paludrine and mepracrine.

Acute attacks of B T respond to single oral doses of as little as 10 mg. paludrine. Doses of 300 and 200 mg mepracrine are also usually effective. A dose of 100 mg mepracrine will not relieve the acute attack.

The plasma concentrations of mepracrine and paludrine after doses of 100 mg and 10 mg. respectively are of about the same order. This may be related to the availability of the drugs to the parasites.

#### 2 Relapse of B T malaria during a course of 100 mg paludrine once weekly

Parasites (B.T.) reappeared in this patient's blood 3 days after taking 100 mg. paludrine (during a regular course of 100 mg. once weekly).

The absorption and excretion of the drug were normal.

This is the only case we have had in which we have confirmed a break through of B T on a regime of 100 mg

#### CASE HISTORY

Age—28

History—P O W Singapore, Malaya, February 1942 to August, 1945.

First attack malaria, August, 1945. Third relapse April, 1946 in United Kingdom. Treated with single dose of 10 mg paludrine. Discharged 3 weeks later on 100 mg paludrine once weekly.

Three weeks later "felt cold, mild shivering, sweating in evenings. Felt vaguely ill for next fortnight and on 25th June, 1946, had rigor and was admitted to Tropical Diseases Centre.

"Paludrine" (ICI).

**Parasites**—B T three asexual parasites per field, rising to ten per field on 28th June  
**Spleen** not palpable  $RBC\ 29 \times 10^6$   $Hb\ 51$  per cent  
**Treatment**—On 28th June, 1946, given 100 mg paludrine Parasites were absent from the blood 3 days later  
 On 4th July, 1946, given 100 mg paludrine At this time no parasites were found in the blood but they appeared 3 days later, and by 11th July there were five B T parasites per field, and the patient was complaining of backache, shivers and his temperature rose to  $99.8^\circ F$

On 11th July he was started on 100 mg paludrine twice weekly and discharged, feeling well, on 20th July  
**Absorption of paludrine**—Plasma concentrations of paludrine were measured after the dose of 100 mg given on 4th July, 1946 Absorption was normal

**Miss Mary Tottey, Dr W H H Andrews, Prof B Maegraith**  
**Distribution of paludrine in whole blood and plasma**

Concentrations of paludrine in whole blood are about four times those in plasma This is due to fixation in the red and white cells as has already been described by TOTTEY and MAEGRAITH (1946) The distribution was determined in blood with normal red and white cell counts The work has now been repeated with two samples of leukaemic blood (One case was myeloid, the other lymphatic leukaemia)

In the cases of leukaemia the ratio  $\frac{\text{amount of drug held per white-cell}}{\text{amount of drug held per red-cell}}$  fell within the range found for normal blood

The results are tabulated on page 368 with an example of distribution of mepacrine in normal blood for comparison

#### "Binding" of paludrine to plasma proteins

It is probably true to say that in the case of nearly all drugs a proportion of the amount present in the plasma is bound more or less firmly to the protein and in such a state is probably not available to the organism to which it is toxic, if this is present in the blood Moreover, only that portion of the drug in the plasma which is not bound in some way to the protein will be free to diffuse into the tissue fluids The destruction of any organisms in the tissue spaces will depend on the concentration in the tissue fluids in the plasma

Such determinations of free and protein-bound paludrine in plasma have been carried out by the indirect method of SHANNON *et al* (1944) In plasma with normal total and differential protein contents the amount of drug bound to the protein is about 75 per cent, that is 25 per cent is in free solution

Whether the albumin or globulin differ in their ability to fix paludrine has not yet been ascertained

#### REFERENCES

- SHANNON, J A, EARLE, D P, BRODIE, B B, TOGGART, J V & BERLINER, R W (1944)  
 The pharmacological basis for the rational use of atabrin in the treatment of malaria  
*J Pharmacol*, 81, 307  
 TOTTEY, M M & MAEGRAITH, B G (1946) Pharmacology of paludrine in human subjects  
*Trans R Soc trop Med Hyg*, 40, 2

THE DISTRIBUTION OF PALUDINE IN NORMAL AND LEUKAEMIC BLOOD  
 COMPARED WITH THE DISTRIBUTION OF MISPACINE IN NORMAL BLOOD

Element.	Paludrine in normal blood.				Paludrine in leukaemic blood				Mispacine in normal blood	
	Experiment 1		Experiment 2		Experiment 1 Lymphatic leukaemia		Experiment 2 Myeloid leukaemia		Normal blood.	
	µg./l. blood	% of blood con- tent.	µg./l. blood	% of blood con- tent.	µg./l. blood	% of blood con- tent.	µg./l. blood.	% of blood con- tent.	µg./l. blood.	% of blood con- tent.
Erythrocytes	821	73	830	79.4	296	20.5	672	12.1	49	13.5
Plasma	184	13.6	147	14.2	244	23.1	227	4.1	1	10
Leucocytes	182	13.4	60	6.4	529	54.4	4 660	82.8	186	76.3
Whole blood	1,127	—	1,033	—	873	—	5 560	—	205	—
W.B.C. per c.mm.	6,400		6,450		70 000		176 000		2 400	
R.B.C. per c.mm.	4 180 000		4,320 000		2,800 000		1 600 000		5 040 000	
Amount drug per W.B.C.										
Amount drug per R.B.C.	120		54		108		62		2 400	

Prof B Macgrath and Dr F J McLean

The mechanism of renal anoxia. Evidence of a renal vascular reflex and the establishment of collateral renal circulation in the rabbit.

The experiments illustrated in the charts showed that after injection of hæmoglobin (which gives rise to obstruction of scattered small vessels mostly in the cortex) reflex constriction of other renal vessels occurs with the production of sufficient degree of renal ischaemia to evoke a Goldblatt hypertension.

In this case the reflex has been initiated from *within* the kidney substance FRANKLIN and his colleagues in Oxford have recently shown that reflex changes in the renal vessels and thus in renal blood flow can be initiated by direct stimulation of the renal nerves

The blood flow through the smaller vessels of the kidney can therefore be affected reflexly by both intra- and extra-renal factors

**The pathogenesis of the renal anoxia syndrome**  
We believe that such changes in renal flow are the basis of the pathogenesis of the renal anoxia syndrome, be it in blackwater fever, crush injury, traumatic shock or cholera

Anuria develops as the result of failure of glomerular blood flow. There is evidence that such failure of glomerular flow is related to a more general reduction in cortical blood flow, the degree of which determines the degree and permanency (or otherwise) of interference with tubular function. The reflexes described above probably play an important part in the production of cortical ischaemia, for example, by opening up large arteriovenous anastomoses capable of diverting the blood flow from the cortex to the medulla (Photographs illustrating this reflex were shown through the kindness of Dr K J FRANKLIN, Oriel College, Oxford)

**Establishment of collateral circulation in the kidney (rabbit)**  
Serial sections of injected rabbit kidneys revealed three types of vessel capable of short-circuiting the glomerular flow. The experiments illustrated show that efficient collateral circulation can be established through such vessels since

- (a) the Goldblatt effect is lost when the collateral circulation is established,
- (b) the Goldblatt effect is restored when the collateral circulation is cut

Collateral circulation is established through existing vessels and it is clear from the normal kidney function resulting from such establishment of collaterals, that the new flow must enter the cortex on the afferent side of the glomerular plexus

Such vessels as these may in the normal kidney form a path for short circuiting blood from the cortex. It is not unreasonable to suggest that similar vessels occur in the human, and are concerned in the genesis of the renal anoxia syndrome

DEPARTMENT OF ENTOMOLOGY AND PARASITOLOGY  
Prof R M Gordon and Dr N M Hancox

Smears and sections showing the primary phase of *P. gallinaceum* at the site of inoculation with sporozoites

An emulsion of sporozoites, from the salivary glands of four infected *Aedes aegypti* was obtained free of bacteria, by using the technique described by GORDON and HILL (1946). This emulsion was then mixed with Chinese ink as a "tracer" and inoculated into the brain of the bird at 12.30 p.m., 12.11.46. The bird was killed and the brain removed at 11 a.m. 16.11.46 i.e. 95 hours later. At this time the site of inoculation was found to be clearly defined by the Chinese ink, and the smears and sections shown were made from the marked area and stained respectively with Giemsa and with haematoxylin and eosin.

The stages of development of the parasites appear to be mainly those described by HUFF and COULSTON (1944) as metacryptozoites.

#### REFERENCES

- GORDON R. M. & HILL, M. A. (1946). *Ann. trop. Med. Parasit.* 40, 113-115.  
HUFF C. G. & COULSTON, F. (1944). *J. infect. Dis.* 78, 231-249.

Dr D. S. Borirarn

Methods employed in investigations on the transmission of *Litaneosoma caribae* by *Liponyssus bacoti*.

Uninfected female *L. bacoti* bred from eggs are allowed to feed over a period of 24 hours on a caged infected cotton rat placed on a tray of sawdust mounted over water. The gorged mites are collected from the sawdust and from a water tray over which the rat in its cage is transferred after the allotted feeding period. Thereafter the mites are stored in tubes at 25° C. and maintained, until infective, by periodic blood meals on the scarified tail of a white rat.

The apparatus used in these methods was exhibited. A result was recorded in which a white rat, exposed to infection by mites feeding on the tail every 4 to 6 days over a period of 33 days, was found to be positive for small numbers of microfilariae in the peripheral blood approximately 3½ months later.

It was concluded that the transmission of *L. caribae* to white rats by *L. bacoti* occurred in connection with the feeding habits of the mite.

Dr W. E. Kershaw (material supplied by Mr H. F. Carter, Ceylon)

Pulmonary ascaridiasis occurring in monkeys in captivity in Ceylon

Three experimental monkeys in the Department of Anatomy at the Ceylon Medical College, and one at the Colombo Zoo, were found to be infected with *Pharyngodon* sp.

The extensive cavitation in the lungs was illustrated by a section, and by radiographs of intact animals and isolated lungs. The isolation of most of the cavities was demonstrated by radiographs following lipoidal injection of the bronchi.

Miss M. A. Hill and Miss E. W. Roberts

The control of adults and larvae of *Culex* with gamma-hexachlorocyclopentadiene

Experiments were carried out at Knowsley Park, Liverpool to estimate

Gamma-hexachlorocyclopentadiene (ICI).

the immediate and the residual effect of gammexane on the larvae of *C. impunctatus* and on adults of *C. impunctatus* and *C. obsoletus*. The breeding ground was sprayed with 100 mg gammexane per square foot and samples were taken immediately. Two days after spraying, 50 per cent of the larvae were dead and none were recovered alive on any subsequent date, thus suggesting that the residual effect was sufficient to prevent the development of the second generation of larvae. Tests made in the laboratory, by introducing healthy larvae to sods taken from the sprayed area, showed that 4 months after spraying 100 per cent of introduced larvae were dead in 7 days, while more than 95 per cent of the controls were alive.

To test the effect of gammexane against adult *Culicoides*, squares of cloth were impregnated with gammexane at concentrations varying from 25 to 520 mg per square foot, the cloths being exposed to field conditions throughout a period of 24 days, during which collections were made every 6 days of adults momentarily resting on the cloths. Over 95 per cent of all adults collected on the first day died within 15 hours, and even on the 12th day over 60 per cent of collected adults died in the same observation period. There was no marked difference between the immediate or residual effect of gammexane at the varying concentration.

### Miss E W Roberts

Slides showing cysts of *Entamoeba histolytica* deposited in the vomit drop of *Musca domestica*

The cysts used in the experiments were concentrated  $\times 4$  from faeces by the method advocated by YORKE and ADAMS (1926). Each fly was enclosed in a rectangular glass box, the slide containing the emulsion of cysts acting as a base. Vomiting was induced after feeding, complete by replacing this infective food by a dried blood film, on which the subsequently cleared area indicated the position of a vomit drop. Of over 50 vomit drops obtained by this method 50 per cent contained cysts of *E. histolytica*, an average of 1.5 cysts being found in each vomit drop. Cysts were found to occur in the vomit drop up to, and not after, 9 hours of feeding, but experiments carried out with organisms of various sizes suggest that although *E. histolytica* cysts occur in the vomit drop, they are not present in the material expectorated from the crop, but are merely washed out by this fluid from the crop to which they adhered during feeding on the contaminated substance.

### REFERENCE

- YORKE, W & ADAMS, A R D (1926) Observations on *Entamoeba histolytica*. *Trop Med Parasit*, 20, 279.

### Dr J F E Bloss

- 1 Photographs of leprosy cases and examples of types of the disease in the Temburu Yambio area of the Sudan. Reference Bloss, J F E (1946) *Trans R Soc Trop Med Hyg*, 40, 1-10.



2 Photographs and maps showing the Sudan Medical Service Hospital  
Steamer the *S IV Lady Baker*

This steamer which is equipped with ward, operating theatre dispensary small laboratory and accommodation for medical staff tours some 1700 miles of rivers in the Upper Nile Province of the Anglo-Egyptian Sudan.

Dr A C Fisher and Dr A C Lendrum (shown by Dr Monica Fisher).

Tropical phlebitis in Northern Rhodesia. Sections of biopsy material.

The condition of primary tropical phlebitis, first described in 1941 is an acute febrile illness with venous thrombosis. The impression had been gained at necropsy that there was but a small zone of primary damage in the wall of the vein and a recent acute case involving the superficial jugular vein at last provided confirmation of this view. The sections reveal a peculiar type of inflammatory tissue composed of actively proliferating capillaries and an infiltration of polymorphs, of unusually well preserved appearance, and large aberrant wandering cells (polyblasts) in the oedematous zone round the new capillaries. This granulation tissue appears to invade and disrupt the tissues of the media, and the thrombosis is presumed to be secondary to the mural damage. In the polyblasts, there are demonstrable intracytoplasmic inclusions with pronounced phloxinophilus. These suggest the possibility that a virus is present, and certainly the type of inflammatory response is quite unlike any of the usual pyogenic lesions. Fuller details will be published later (FISHER, A. C., FISHER, MONICA M. and LENDRUM, A. C. *J Path. Bact.* In press).

FISHER, A. C. (1941) *South African med. J.* 14, 131 GILFORD, M. (1941). *The Sick African*, Cape Town.

Dr P A Garnham (shown by Professor Shortt)

An unusual case of *Plasmodium falciparum* malaria in a member of a highly immune tribe showing very numerous schizonts in the peripheral blood.

The case occurred in an adult African of the highly immune Loo Tribe. Although the temperature rose to 105° F for a few hours the patient did not appear to be very ill and the attack responded rapidly to oral quinine. Cerebral symptoms were absent.

Dr C J Hackett.

Photographs of *Aedes aegypti* in flight.

Six photographs of *Aedes aegypti* in flight taken by Mr W H WALTON were shown by courtesy of the Chief Superintendent, Chemical Defence Experimental Station, Porton. These were made by condenser discharge flash of one microsecond's duration actuated by the insect in flight passing through the intersection of two beams of light. The other equipment was of normal harmonic

Dr F Hawking, Mr R Hunt and Miss P Davey

Demonstration of laboratory cultivation of *Anopheles quadrimaculatus*

A method of cultivation was shown, based on that described by HEAL and PERGIN (1946) \* By this means 3,000 to 4,000 female mosquitoes can be produced every 12 days It is hoped shortly to publish a full account of this technique

\* HEAL, R E & PERGIN, M M (1946) *Proc 32nd Ann Meeting of the New Jersey Mosquito Extermination Association*, March

Dr K B Rogers (shown by Mr P G Shute)

A case of quartan malaria (*P malariae*) brought about as the result of whole blood transfusion

A female patient, aged 19, who had never been out of England, was given two pints of whole blood transfusion for ante-natal anaemia on 8th March, 1946 The blood had been stored for 4 days Six days later she had a normal labour and gave birth to a healthy male child At the end of April she began to feel off colour but did not call in her doctor Severe sweating at night was the chief feature but it is not known if any rigors occurred before the one on 15th June (after admission to hospital) which recurred every third day Blood films examined on 26th June contained *P malariae* parasites

The stored blood was from two donors, a man and a woman No parasites were found in the woman's blood but a male quartan parasite was found in the man's blood He was a ship's engineer, and in 1939 travelled through many endemic malarious regions including the Mediterranean, Aden and Basra This was his only foreign service and he was abroad for not more than 5 months As far as he knew he had never had malaria but complained of "cold turns" after his return to England at the end of 1939 When parasites were found in the blood of the donor a few c c were injected into a case of G P I awaiting malaria-therapy Fever and parasites occurred a few weeks later The blood of the child was examined for parasites on one occasion but none was found

Mr P G Shute

A case of latent *P ovale* with a spontaneous recovery

A service man (R A F) went to West Africa in June, 1943, and was stationed at Bathurst and Stradishall where he remained until May, 1946 This man returned to England at the end of May, 1946, and on 3rd July he was admitted to hospital with a temperature of 105.8° F *P ovale* parasites were very scanty in a thin film, not more than 10 per c mm No anti-malarial drugs were given for 5 days but despite this there were no further attacks of fever and parasites could not be found in either thick or thin films The patient states that he did not have a single day's illness while abroad, and it is considered that this is a case of true latency, probably due to mepacrine suppression

The patient had been taking two tablets (0.2 gramme) of mepacrine twice

weekly beginning 7 days before arrival in West Africa and later this was changed to one tablet daily (0.7 grammes a week). This continued during his stay in West Africa and for 2 months after returning to England. His stock of *P. ocale* developed within a short time of discontinuing mepacrine.

Mr P. G. Shute and Mr E. Ungureanu

A means of identifying the varieties of *A. maculipennis* adults by the shape and size of the scales of the wings.

It is over 20 years since it was found that there are several varieties of *A. maculipennis* within the group and that they can be identified by the surface pattern of the egg and also in the males by the shape of the external spine of the hypopygium. Recent observations by one of us (E. U.) afford a means of identifying at least some of the varieties of the female adults by the shape and size of the scales of the wings.

*Atroparvus*.—The scales are slender and taper gradually towards the tip.

*Messiae*.—The scales are wider than in *atroparvus* and taper acutely towards the tip.

*Typicus*.—The scales are wider than in *messiae* and they taper less acutely towards the tip than they do in *messiae* but more acutely than in *atroparvus*.

*Eltis*.—The scales are widest in the centre and taper gradually towards the tip but more acutely than in *atroparvus*.

*Labranchei*.—The scales of the wings of *labranchei* are identical with those of *atroparvus*.

Dr J. C. Broom

Simons's stain (technique with thodane blue solutions T.3 and T.5).

T.3 and T.5 are aqueous solutions of a compound of methylene blue and saponin.

The solutions haemolyse red blood cells instantaneously and stain parasites which are not affected by the saponin, leucocytes and reticulocytes. The method is useful for demonstrating parasites in scanty infections as they are more readily seen in the absence of red cells.

*Technique*.—With T.3 two loopfuls of stain are placed on a slide and one drop of blood, taken with the wet loop, is mixed with the fluid and examined immediately under a coverglass with a 1/16 inch objective, using either bright or dark field illumination.

With T.5 one drop of solution is placed on the slide and four or five drops of blood added, giving an "ultra thick drop."

The demonstration showed trypanosomes in mouse blood and leptospirae (dark field) in guinea pig blood stained by this method.

#### REFERENCE

Simons, H. C. R. (1946). *Schweizer Med. Woch. 76*, 607.

## Mr C B Symes

- 1 Charts were shown containing preliminary data from one of the field experiments now being conducted by the Colonial Insecticide Research Team, in Uganda

Essential portions of the data are reproduced in the table below

- 2 Two of the models of mosquitoes, copied in wood by native craftsmen from plasticine originals, have been used for some years for teaching purposes in the section of Medical Entomology, Kenya

TABLE  
KASENJI EXPERIMENT

	Sabadu (control)	Mum- vuka	Saba- wali	Mutuba II	Mutuba III	Musale	Saba- gabo
Number of houses	793	446	403	141	305	485	459
Treatment applied	None	5% DDT in diesoline	5% gam- maxane (D930) in diesoline	5% gam- maxane (P530) in water (powder)	5% DDT in kerosene and cotton- seed oil	walls only 5% DDT in diesoline	roofs only 5% DDT in diesoline
Weekly average of vector* mosquito samples for 8 weeks before treatment	6	36	8	82	78	17	44
Parasite rate in children in May, 1946 (before treatment)	20.1	30	35	54	51	35	27
Weekly average of vector mosquito samples for 5 weeks after treatment	8	0.4	0.4	2	0.6	0	0
Parasite rate in children in August (3 months after treatment)	29.8	27.7	22.9	25.4	49.4	32.5	44.3

\* Vectors were *A. gambiae* and *A. funestus*

## Mr F L Vanderplank

## Apparatus for feeding blood-sucking insects in the laboratory

The chief difficulty of keeping various blood sucking insects in the laboratory is one of labour involved in feeding them. Various forms of apparatus have been used in the past to maintain such insects in captivity. All these

appliances seem to have various snags when used to feed tsetse flies. Tsetse flies will feed through a skin membrane fairly readily provided the liquid below is warm and saline.

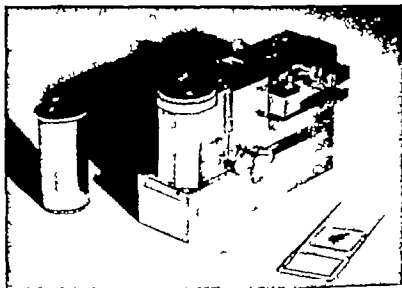
It is desirable to have membrane above and liquid below so that a fly can be shaken down the tube on to the membrane. If this procedure is reversed it is often difficult to get the fly in contact with the membrane. The requirements of any feeding apparatus are, therefore, a membrane with liquid immediately below it and without any intervening air bubbles and this liquid should be maintained at a constant temperature. The apparatus demonstrated consisted of shallow feeding dishes made of perspex, and with a rat skin membrane stretched across the top. Blood or other liquids were circulated below. Circulation carried out by means of a small centrifugal pump made entirely of perspex and all operated by an electric motor. Blood was heated by passing through a glass tube surrounded with water electrically heated and thermostatically controlled to the desired temperature. Filling the system with liquid and getting rid of air bubbles was done by means of a single vertical tube with an inlet at the top, outlet at the base. Whole blood prevented from clotting by addition of leach anti-coagulant has been found to be most suitable to maintain the tsetse flies. Defibrinated blood also proved successful. Citrated blood was found not to be so suitable. Tsetse can be induced to feed on almost any liquids but they dislike plain water. They will feed quite readily on any solutions containing saline. When blood is used in such an apparatus great care should be taken that it does not come in contact with any metal parts, otherwise its composition is rapidly altered by electrical action of the metal in contact with the blood. The blood should also be reasonably sterile since excessive bacteria have harmful effects on the flies. After use, the skin membranes can be preserved in 70 per cent. alcohol and last up to 1 month when used daily. This apparatus has been used to feed mosquitoes and tick beardes glauca and it is hoped to publish a full description later.



Dr John McArthur

A new type of research microscope

A microscope was shown which was claimed to be capable of any work normally undertaken by a conventional research microscope, but with certain additional and unusual abilities of its own. While carrying standard optical equipment, mechanically it is a complete departure from conventional design, and bears no resemblance to the ordinary microscope.



This microscope measures only  $4 \times 2\frac{1}{2} \times 2$  inches and weighs just over 2 lb but apart from its small size enormous rigidity and convenience of handling, which make it an excellent travelling instrument, it has further advantages by which it challenges the position of the conventional research microscope for example automatic focusing erect image free access to the object, for staining and microdissection, even while under observation by an oil immersion objective and simplified operation. Facilities are provided for a full range of accessories.

A full description of the instrument will be published in a forthcoming number of the *Journal of the Royal Microscopical Society* (probably Vol. LXVI Nos 1 and 2).

ORDINARY MEETING  
of the Society held at  
Manson House, 26, Portland Place,  
on

Thursday, 12th December, 1946, at 8 p.m.

THE PRESIDENT,  
C. M. WENYON (M.C., C.B.I., M.B., B.Sc., F.R.S.),  
in the Chair

PAPER.

COLONIAL NUTRITION AND ITS PROBLEMS

BY  
B. S. PLATT, M.C., M.Sc., PH.D., M.B., CH.B.  
*Director of the Human Nutrition Research Unit of the Medical Research Council*  
*Professor of Nutrition, University of London*

On two occasions recently I have talked about colonial nutrition, at a Nutrition Society conference on Colonial Nutrition (PLATT, 1946a) and at a discussion during the Royal Society Empire Scientific Conference (PLATT). I shall, however, try to avoid repetition as far as possible and this evening emphasize the medical aspects of the problem.

Ten years ago a general survey was made of the nutrition of colonial peoples by a Sub-Committee of the Economic Advisory Council. Part II of the report of this Sub-Committee summarizes the information received from the various colonial territories on (i) dietaries and their relation to the state of nutrition, (ii) practical measures for improvement of nutrition and researches, and (iii) practical measures for further investigation. Part I of the report was prepared as a guide to the nutritional health of colonial peoples, and as a stimulus for further investigation. I want to draw attention now to certain information relating to the nutritional health of colonial peoples in the decade 1937-1946, to give you examples of investigations into the prevalence of some signs of nutritional ill health and their relation to the nutrient content of the foods eaten, and then to describe briefly the lines on which we are working for the improvement of the nutrition of colonial peoples.



PREVALENCE OF NUTRITIONAL DEFICIENCY DISEASE AND DISEASE PARTLY  
ATTRIBUTABLE TO MALNUTRITION.

Some of the most recent figures showing the occurrence of diseases due to dietary deficiencies are shown in Table I.

These data are drawn from annual reports of medical departments. I wish particularly to remind you that the figures refer to patients seeking treatment at hospitals or dispensaries—they leave out of account the sick who do

TABLE I  
OCCURRENCE OF NUTRITIONAL DEFICIENCY DISEASES IN COLONIAL TERRITORIES (41 IN ALL)  
1928-1943.

Disease	Territories reporting.	Notes.
Beri-beri	20	Hongkong 13 644 cases, 7,009 deaths, 1940 Malaya 1 602 deaths, 1939
Pellagra	24	Basutoland 807 cases, 1942. Hongkong 823 cases 442 deaths, 1940. Nigeria 413 cases, 1942.
Stomatitis ( )	8	Nigeria 3 385 cases, 1938. Uganda 1 807 cases, 1938 Mauritius 1 033 cases, 1938.
Scurvy	17	Bechuanaland 610 cases, 1939 Tanganyika 167 cases, 1935. Switzerland 136 cases, 1942.
Rickets	13	Ceylon 643 deaths, 1944
Goitre ...	4	High incidence Upper Gambia, 1943; prevalent, N. Nigeria, Cameroons, 1942
Nutritional (b)	13	Ceylon 1 329 deaths, 1944, 83.7% cases of multiple A and B deficiency 1944 Sierra Leone 2,153 cases, 1942. Nigeria 2,046 cases, 1942. Gold Coast 1 326 cases, 1944 Uganda 751 cases, 1939

(a) Probably mainly due to B<sub>12</sub> complex deficiency.

(b) Nutritional diseases of unspecified origin reported in the diseases of nutrition, endocrines and rheumatism.

Tables I to V are taken from a paper read by Professor PLATT which will be published later in the *Proceedings of the Royal Society*.

not reach the doctor and, more important still, they provide only an indirect indication of the extent to which malnutrition occurs in the population generally

The main observations on these data are —

(1) Beriberi is still a common disease and cause of death in Hongkong and Malaya

(2) Pellagra is a problem in Hongkong, Nigeria and the Gold Coast, and recently in Basutoland

(3) Scurvy is not uncommonly diagnosed in Bechuanaland, Tanganyika, Northern Rhodesia and Nigeria

(4) Rickets is reported from Nigeria and Aden in fair numbers, it is given as a cause of 605 deaths in Ceylon in 1944

(5) A large number of diagnoses of nutritional deficiency disease of unspecified nature occur in the returns of medical departments, and not infrequently this is given as a cause of death, for example, sixty-nine in the Gold Coast in 1944, seventy-two in British Guiana in 1943, and ninety in St Vincent in 1943

In Table II are recorded recent data on the occurrence of five diseases which, in part at any rate, are due to poor nutrition

TABLE II  
OCCURRENCE OF DISEASES PARTLY ATTRIBUTABLE TO MALNUTRITION IN COLONIAL TERRITORIES  
1936—1945

Disease	Territories reporting	Notes
Anaemias	14	Mauritius 6,774 cases, 1938 Nigeria 6,632 cases, 1938 Cyprus 2,883 cases 1938 Kenya 1,352 cases 1937
Tropical ulcer	16	Nyasaland 28,029 cases, 1944 Uganda 26,365 cases, 1936 Gold Coast 15,921 cases, 1938
Infantile diarrhoea	27	Nigeria 4,292 cases, 1943 Kenya 3,610 cases, 69 deaths, 1937 Gold Coast 3,109 cases, 1944 Uganda 2,634 cases, 1939
Toxaemia of pregnancy	15	Nigeria 1,729 cases, 53 deaths, 1943 Sierra Leone 479 cases, 1943 British Guiana 19 deaths, 1943
Tuberculosis of the respiratory system	38	Hongkong 5,751 deaths, 1940 Malaya and S S 4,911 deaths, 1939 Ceylon 3,141 deaths, 1944 Jamaica 1,520 deaths, 1943

The prevalence of tropical ulcer is especially high in many territories patients suffering from this disease are so numerous that temporary structures have to be added to hospitals to accommodate them. A surgeon in a district in East Africa told me in 1938 that 90 per cent. of his time spent on operations was on skin grafting in the treatment of ulcers.

### INFANT AND MATERNAL MORTALITY RATES.

The data available on infant and maternal mortality are inaccurate but, even if the errors are large they show that there is need for great progress in maternal and infant welfare.

TABLE III.  
INFANT MORTALITY FOR COLONIAL TERRITORIES. 1936-1943  
(The most recent figures available)

	Deaths per 1,000 births.		Deaths per 1,000 births.
United Kingdom	46	Dominica	117
Seychelles	56	Bahamas	119
Fiji	63	Gold Coast (c)	129
St. Helena	63	Gambia (d)	130
Virgin Islands	63	British Honduras	130
Bermuda	68	Malaya and Straits Settle- ment	133
S. Kitts Nevis	76	Ceylon	133
St. Lucia	80	British Guiana	141
Cyprus	82	Mauritius	163
Palestine	87	Barbados	164
Montserrat	88	Serra Leone ( )	187
Nyasaland (a)	95	N. Rhodesia (f)	191
Jamaica	98	Aden	197
Antigua	99	Malta	216
Tonga	101	Tanganyika (g)	243
Gibraltar	104	Gibraltar and Ellice Islands	244
St. Vincent	106	Nigeria (h)	250
Trinidad	109	Zanzibar	280
Nigeria (b)	113	HongKong	337
Uganda	116		

Note.—For eight territories, there are no figures available.

( ) Eleven villages Kaituma district.

(b) For Lagos only.

(c) For colony only.

(d) For Barbados only.

( ) For Freetown only.

(f) Chumbale area only.

(g) A rough estimate.

(h) Approximate for whole country.

The Chief Medical Officer of Health (United Kingdom) reports (1944) that both infant and maternal mortality rates in this country are now at record

low level. This is in spite of war conditions, and may well be related to the improved feeding of mothers. Contrasted with this report is, for example, one from Mauritius (1945), showing an increase in infant mortality rates from 141 to 188 per 1,000 live births, and in maternal mortality from 8.73 to 14.96 deaths per 1,000 births.

TABLE IV  
MATERNAL MORTALITY FOR COLONIAL TERRITORIES 1936-1945  
(The most recent figures available)

	Deaths per 1 000 births		Deaths per 1 000 births
United Kingdom	2.8	Malaya and Straits Settlement	9.0
St Vincent	3.0	Nigeria (a)	9.25
Hongkong	3.5	Seychelles	11.0
Bahamas	4.25	Cyprus	12.0
Mauritius	5.0	Malta	13.25
Uganda	6.25	Ceylon	13.5
Fiji	8.0	Gold Coast	16.0

Note.—In 33 of the 44 territories there are no figures available  
(a) For Lagos only

### INFANT BIRTH WEIGHTS

One of the most interesting nutrition investigations in recent years has been concerned with the state of nutrition of the newborn infant and the occurrence of complications during pregnancy, labour and delivery in relation to the nutrition of the mother during pregnancy (BURKE, HARDING and STUART, 1943). For example, the birth weight of an infant is related to the total protein in the mother's daily diet during pregnancy, thus boys' and girls' weights increase from an average of 6 lb 8 oz and 5 lb 14 oz to an average value of 9 lb 2 oz and 8 lb 8 oz respectively, according as the daily diets of their mothers contained under 45 grammes or 85 or more grammes of protein.

Table V on page 384 shows how the average birth weight increases with the social status of the mother and that the birth weights of Asiatic and African infants of poor mothers correspond with those for infants of American mothers at the lowest level (less than 45 grammes) of protein intake per day.

### EVIDENCE OF MALNUTRITION FROM CLINICAL RESEARCHES

I should like to remind you of investigations which have been made in the past decade in many colonial territories, notably Ceylon, Uganda, Nigeria, Tanganyika, Malaya, Mauritius, Hongkong and the West Indies. You will

TABLE V  
BIRTH WEIGHTS IN THREE COLONIAL TERRITORIES.

<p>Nyasaland—rural area.</p> <p>A crage weight of 105 male infants 6 lb. 11 oz. 118 female infants 6 lb. 8 oz.</p>		
<p>Malaya—Kedah estates and Penang.</p> <p>Average weights of infants (both sexes) born in hospital to patients of different social groups, and different races.</p>		
Social and racial group	A crage birth weight.	Number of observations
European paying (Penang)	8b 3½ oz.	125
Chinese paying (Penang)	6 lb. 11½ oz.	379
Indian paying (Penang)	6 lb. 5 oz.	108
(Kedah)	6 lb. 4½ oz.	29
Chinese non-paying (Penang)	6 lb. 6 oz.	2,639
Indian non-paying (Penang)	5 lb. 12 oz.	845
(Kedah)	5 lb. 11 oz.	667
<p>Ceylon—Colombo hospital.</p> <p>A crage weight of Indian and Sinhalese infants born in hospital to patients of different social groups</p>		
Paying patients	6 lb. 14½ oz.	18
Non-paying patients	6 lb. 0 oz.	—,033

no doubt immediately associate names with work on malnutrition in these territories, the results of many of which have been reported to the Society. These investigations demonstrate beyond doubt that there is disease due to malnutrition in colonial territories.

Recently (Dr WATERLOW 1946) a member of the staff of the Human Nutrition Research Unit, has investigated a number of cases in the West Indies of what I have called "sugar babies." These infants which have been fed on a diet high in carbohydrate and low in protein have grossly fatty livers, extremely wasted skeletal muscles and, generally oedema. They have, however only minor changes of the skin and hair thus differentiating them in part from the kwashiorkor of CICELY WILLIAMS or the malignant malnutrition of TROWELL. This fatty liver disease can be cured by feeding milk, the effect being attributable to milk protein. It seems from a consideration of the

results of this investigation, together with other observations on man and on experimental animals, that some dietary deficiencies may give rise to liver cirrhosis and possibly to such impairment of liver function as, in time, to affect the utilization in the body of nutrients. It is important to appreciate that the effects of disease, whether due primarily to malnutrition or to zymotic factors, may in various ways modify the requirements of the body for nutrients.

### MALNUTRITION IN THE GENERAL POPULATION

I do not need to stress before this Society the difference between estimates of the prevalence of disease or of the state of health in colonial territories determined from hospital returns, and those obtained from surveys of samples of the general population. Whilst the kind of data I have already presented may provide some indication of the nutrition problem, a satisfactory measure of its nature and dimensions can only be obtained from surveys for evidences of nutritional ill-health, preferably combined with a study of food consumption, of food supplies and of various factors likely to affect the food economy.

The method of assessment of the state of nutrition of man has been discussed many times, and various indices and devices have been invented to assist in making assessments. In China, in 1935, I started to record such signs and symptoms of nutritional ill-health as could be obtained on a simple clinical examination, and continued to use this procedure during the past 10 years. I admit that there are still many difficulties in interpretation, and these I have considered elsewhere (PLATT, 1944 and 1945).

### PREVALENCE OF SIGNS OF NUTRITIONAL ILL HEALTH IN THREE COMMUNITIES

I have selected from a number of investigations three sets of data showing the prevalence of a few only of the minor signs of nutritional ill health. The first is a group of Barbados rural and urban school children examined in 1944 (see Figure, page 386). Signs are common in hair, eyes, tongue and skin, as well as dental caries. Half to two-thirds of the children had one or more of these evidences of malnutrition which in general are the results of insufficiency in the diet of protein (or more correctly possibly, of some amino acids) and of vitamins of the B<sub>2</sub>-complex.

The incidence of these minor defects was similar in a group of rural children recently examined in the Gambia (see Figure).

The data shown for Nyasaland are the combined results of examinations of the inhabitants of three rural villages and of a number of families in an urbanized area. Again the same manifestations occur, but many of them are of more frequent occurrence than in the other two areas (see Figure).

For comparison, I may quote the results of systematic clinical surveys conducted by the Ministry of Health, during which, of 20,235 people examined,

## PREVALENCE OF SIGNS OF NUTRITIONAL ILL HEALTH IN THREE COMMUNITIES

SIGNS OF NUTRITIONAL ILL HEALTH		BARBADOS 192 SCHOOL CHILDREN	GAMBIA 145 VILLAGE CHILDREN	NYASALAND 474 ADULTS & CHILDREN
		2	7	7
HAIR	dry starting	8	34	16
	hypochromotrichia	22	84	
EYES	photophobia	(1) 20	39	10
	excess tissue	65		33
LIPS	cheilosis	7	17	
TONGUE	colour changes	43		
	papillae lost	6		7
	fissures	5		6
GUMS	bleeding	1	3	42
TEETH	curles	44	27	29
SKIN	atrophic	0		63
	defective hairs	6		19
	xerosis	52	63	74
	crackled	14	(11)	85
	loss of elasticity	54	37	
	permanent gooseflesh	7	0	43
	folliculosis	19	17	12
	ulcers and scars	30	37	53
NAILS	defective or rough	1		34
MUSCLE	poor development	7		52

(1) EARLY STAGES

(11) 54% AFFECTED IN 50 CHILDREN EXAMINED IN 1945

"only nine showed evidence of deficiency disease, only 0.7 per cent were of 'poor,' and only 10.5 per cent of 'fair' nutritional state" (MAGEF, 1946)

#### NUTRIENT CONTENT OF DIETARIES FOR THREE SELECTED COMMUNITIES

Before looking at the nutrient content of the dietaries of the sample communities, may I give you the levels for various nutrients which may be considered reasonable and desirable for the population of colonial territories (PLATT, 1946c). The values are given on a per head basis for a population of mixed age and sex groups (Table VI, column i)

It is of interest to compare these with the amounts supplied in our diets in the United Kingdom in 1944 (Table VI, column ii)

In reading the amounts (Table VI, column iii) in the diet of the Barbadian (PLATT, 1946c) it should be noted that these values are only an indication of

TABLE VI  
NUTRIENT CONTENTS OF DIETARIES.

	Values recommended as an immediate objective for populations of colonial territories	Nutrients available per head per day						
		U K 1944	Barbados 1944	Gambian village 1945	Nysaland survey, 1938-39			
					Hill village	Foot-hill village	Lake shore village	Urban area
	i	ii	iii	iv	v	vi	vii	viii
Calories	2 500	2,923	2,413	2,028	1,805	2,049	1,739	1,655
Protein (grammes)	60	87	45	50	50	59	20	54
Fat (grammes)	—	117	63	25	15	16	9	11
Carbohydrate (grammes)	—	381	416	396	357	400	387	334
Alcohol (mg)	—	—	—	—	2.5	8.6	1.4	0.8
Calcium (mg)	800	1 037	254	219	280	470	1,005	702
Iron (mg)	20	16	12	26	25	34	21	24
Vitamin A (i u)	5,000	3 773	5,215	3,536	7,757	10,220	6,020	6,914
	(as $\beta$ carotene)							
Vitamin B <sub>1</sub> (mg)	1.5	2.0	0.8	1.8	0.8	1.2	0.7	0.8
(aneurin, thiamine)								
Riboflavin (mg)	1.8	2.1	0.8	0.5	0.5	0.7	0.5	0.6
Nicotinic acid (mg)	12	19.7	7.0	14.5	9.0	11.5	9.6	7.0
Vitamin C (ascorbic acid) (mg)	30	123	69	28	80	98	120	73



individual intakes because they are calculated from total food supplies and it is recognized that there are inequalities of distribution. Food consumption data obtained from dietary surveys of individuals or small groups give values about 10 per cent. less for nutrient intake than when obtained from data on gross food supplies. Compared with the levels of requirements I have given, there are outstanding deficiencies of protein, B-vitamins and calcium.

The values for a sample Gambian village compound were obtained by weighing the food eaten. The data given in Table VI column iv are from our dietary surveys made at 3-monthly intervals throughout the year (Gambia Preliminary Survey 1945-46, DOUGART).

They reveal deficiencies in nutrient intake similar to those of the Barbados dietary—the low intake of calories should be noted and the low value for vitamin A, which is practically all carotene and not preformed vitamin.

The Nyasaland data have again a similar pattern (Table VI v-vm) and are taken from dietary surveys in 1938-39 (PLATT 1947) extending over 10 months in the villages and 1 month in the urban area. On the whole, the Nyasaland diets are poorer in the B<sub>2</sub>-vitamins than those of the other two groups. The protein in the three groups with values of 50.59 and 54 grammes per head per day was very largely from maize, which has been known for many years to be particularly deficient in the amino-acid tryptophan. Some of the protein of the Lakeshore village diet, and most of the calcium, were derived from fish. The calorie value of these four diets is also remarkably low. In none of these evaluations of nutrient content have allowances been made for losses in cooking.

Perhaps these examples will suffice to indicate the state of nutrition of widely separated and representative groups of colonial peoples as judged by clinical evidence and supported by investigations into food consumption. These groups, and undoubtedly many like them, are not getting enough food to enable them to do a good day's work, nor are they getting enough of some nutrients to maintain them in a reasonably satisfactory state of health—and resistance to disease. What can be done to improve this state of affairs?

#### PLANNING TO IMPROVE THE NUTRITION OF COLONIAL PEOPLES

At the Royal Society Empire Scientific Conference (PLATT), it was recommended that essential food factors—vitamins and food concentrates—should be made available so that colonial peoples having frank deficiency disease could be treated, and suffering and loss of life thereby avoided.

I have shown in discussing measures for the improvement of nutrition in the British West Indies (PLATT 1948b) how certain steps as, for example, increasing the nutrient content of wheat flour can be taken immediately in communities which have an economy organized to be able to apply measures of the kind we have used ourselves in our war time food control.

In the more primitive communities, the major problem, to use the words of Sir PHILIP MITCHELL in a recent despatch to the Secretary of State for the Colonies (1946)

"can be stated simply and plainly by saying that in ignorant man and his wife with a hoe are a totally inadequate foundation for an enlightened society, a high standard of living and elaborate social services, and that unless an alternative foundation capable of bearing these things can be devised or, when it exists, can be expanded a great deal of modern talking and writing about colonial development and welfare is moonshine."

I have given elsewhere (PLATT, 1946a) a summary of the various factors which may have to be considered in attempts to increase the amount and availability of food supplies. The improvement of nutrition is certain to be a complex procedure, it involves the co-operation of various departments and agencies, it needs to be carefully planned and will be effective only by the combined efforts of the producer, consumer, technical and administrative personnel, as well as the medical man. It is to be hoped that "combined operations" will be undertaken throughout the Colonial Empire, applying suitable incentives, modern methods of food production and technology, improved hygiene and medical care and education.

Slides illustrating various signs of nutritional ill health were shown, some of those affecting the skin have already been published (PLATT, B S, 1945)

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## DISCUSSION

Dr W. A. Young Mr President, if I put to Professor PLATT a few questions as to the possible "tie up" of other factors with nutrition in producing many of the conditions he has referred to tonight, it is not with any wish to detract from the implication he has made that *the* great need in the Colonial Empire is an improvement in nutrition. It is rather that I don't like to lose sight of the other if minor factors.

Professor PLATT mentioned tropical ulcer. In 1932-33 I witnessed an extraordinary epidemic of tropical ulcer in Zanzibar and Pemba. This corresponded with a year of marked poverty and malnutrition. When, however I worked out the seasonal incidence in the area for a 10-year period, taking a large number of medical institutions into my calculations, I discovered a definite seasonal rise in January February and another about October and this seasonal variation I could in no way relate to any nutritional conditions. In vain I set forth all the food crops and investigated the stores of foods in the villages at the different times of the year. I started off, therefore, believing I had tropical ulcer related to malnutrition, but failed to explain on these grounds the definite seasonal variation in incidence. I remember I showed this to Professor PLATT and that he advised me to try to relate my figures to the bean crop. I am afraid I was unable however to convince myself that there was any relation. I came to suspect an infective factor contained in the soil and that this gained entry into the peasant during the seasons of field work.

While on the subject of the infective factor I recall a case I saw the other day of a follicular keratosis. Several people to whom I showed the patient dismissed the case with the pronouncement "pityrioderma." There were, however, no other signs of malnutrition and I believe that this was a case of one of the ideas, a tuberculide or more probably a dermatophytide. Nowadays, in the individual case in Africa we are inclined to exaggerate the part played by nutrition, and to ignore the other factors.

Of the other factors, I should like to ask Professor PLATT if he believes any endocrine adjustment by the individual or the race takes place towards food conditions. In this last war I saw a number of cases of a sort of scurvy among East African troops in an arid part of Kenya. All the patients belonged to one particular people, the Luo who in their homeland enjoy a rich provision of meat, vegetables and milk. I saw no case among Somalis though I believe Somalis were subjected to similar conditions. Surely this was a matter of endocrinal adjustment in some way? Professor PLATT mentioned kwashiorkor. The other day I saw a number of children on the borders of Ruanda who were suffering from malnutrition. There was achromotrichia and a curious straightness of the hair. I at first thought of the Hamitic blood in this part of Africa but the mothers said the hair of the children had gone

like that after a definite illness. How does nutrition come to affect so well recognized a racial character as the cross-section of hair?

Professor PLATT showed us tables of weights at birth in different races and under varying standards of nutrition. Years ago I compared the weights and heights of African, Arab and Indian school children. I found myself unable to determine whether differences in diet or differences of race accounted for the characteristic group figures. It seemed that the Indian ceased to grow at an earlier age than the European child. How does the racial hormonal system reflect the food conditions of a people?

I am afraid I have been a little abstruse. I do realize that, unless we take him in hand, the primitive African may pass out from the modern world as did Neanderthal man with the ice ages.

**Dr J B Davey** I should like to associate myself with the PRESIDENT'S eulogy of Professor PLATT's very interesting paper. I can only speak for East Africa. We have been waiting with some impatience for the report of his survey going on in Nyasaland, but tonight we have been able to enjoy some of the fruit of it. His figures seem to confirm the anxiety felt by those who have worked in East Africa about the nutritional state of the natives there. It has seemed to some of us, to those who are not experts, that previous investigations have been too much concerned with specific deficiencies in the diets—of salts or vitamins. Professor PLATT's investigations confirm Dr RICHARDS'S work in Northern Rhodesia on the Bemba people, from which I may quote. It says the average consumption of calories per day was only 1,706 for adults during 8 months of the year, and in February and March it was only 816. The report shows gross deficiency of animal protein, and Professor PLATT's figures confirm that largely. It seems to me there are three outstanding facts as regards the problem in East Africa. The first that there is widespread and severe under-nutrition at the present time. Those of us who examined a lot of young recruits during the recent war were struck by their miserable half-starved appearance, and actually the weight standard of recruits had to be reduced to 8 stone so as to get enough men. The second point is that, in the past, there was a high standard of nutrition. Many early travellers say what fine people the natives were physically, and those of us who examined natives for the Witwatersrand mines in the early part of the century can certify that they were fine strapping fellows. The third point is what the army has made of these miserable half-starved recruits. In a very short time on Army food, with plenty of meat, those men were practically unrecognizable. This point is of some importance in arriving at an opinion as to the previous state of affairs. There have been various Commissions investigating these matters, and they all agree on two points. One is the lack of good class protein, and the second is the gross insufficiency in the total amount of food. These are

unly understandable facts. I can quote from the local committee's statement Tanganyika "Serious as famines may be, the recurrent annual shortage of food is a much more serious matter." In Tanganyika and other parts of Africa there is such a recurrent shortage. To some of us not nutritional experts it has seemed that other investigators have devoted undue attention to certain specific deficiencies and perhaps have not paid sufficient attention to the fact that people were not getting *enough* to eat. The gross specific deficiencies occur in institutions, that is to say where the food is provided for the native by Europeans in gaols, schools, estates and mining camps. The classical example is perhaps the pellagra in the Central Gaol in Zomba, which more than 30 years ago was worked out, explained and accurately described by a former Vice President of this Society Dr STANNUS. In other words the Europeans did not know how to feed the native. But we know that the native, when he can get the food, knows how to feed himself because in old days he produced a fine type of men who were the admiration of travellers and those of us who examined people for the South African mines. How has the present state of affairs arisen? As regards the protein lack, I would quote from Lord HAILLY's *An African Survey* "In most territories the Africans have looked to wild animals as a sure supply of meat, and in areas where the presence of tsetse fly precludes the keeping of cattle or game meat was, and is still, a necessary addition to diet." Most of these East African territories are overrun by tsetse flies, which makes the keeping of cattle impossible, so that the people are to have meat it must come from game. Then there is a total shortage of food, and here there are various factors concerned. One very important one, which does not receive adequate consideration, is the damage to native gardens by game. The annual reports by Provincial Commissioners and Agricultural Departments abound with references to the gross damage done to native crops by game but I shall only quote this from *The Report of the C (1942) Southern Province Tanganyika*. It says "Vermin, in which term are here included elephant, hippo, and other game continue to take a heavy toll of the African's crops." There are abundant references to that, saying that a large part of the African's crops is destroyed by game. That is the present state of affairs. I would like to ask Professor PLATT if he can tell us anything about the serious damage which has been caused in recent years to native crops in Nyasaland by game, to which the Secretary of State referred in the House of Commons on 3rd April, 1946 and what the Government is doing about it? It seems to me that the present position regarding East Africa is a standing reproach to this country and to our colonial governments. Yet, while this is going on, there is a constant clamour in this country for more game preserves and game parks. Prof. JULIAN HUXLEY in *The Times* of 18th February 1946 says that in Kenya abundant game reserves are needed in addition to national parks. Not long ago the

Secretary of State for the Colonies said that the game reserves in Kenya amounted to 15,830 square miles and those in Tanganyika to about 24,000, or a total of 39,830 square miles. Scotland's total area is 30,410 square miles, so that in these two countries alone we have devoted to game a considerably larger area than the whole of Scotland, and naturally the game stray out of the reserves on to the cultivated land beyond. I happen to have lived in a game reserve, and I know what it means to a native to live in a game reserve. He is not allowed to kill the game within or without a game reserve. It is well known that the Union of South Africa have a problem because of the game in their country, and that Southern Rhodesia has been faced with a similar problem. They are self-governing colonies, not to be deterred by sentiment at home. They are going to deal with the problem, and I should like to ask Professor PLATT if he knows what the colonial governments are going to do to afford Africans that protection from game which the farmers of South Africa and Southern Rhodesia have demanded and are obtaining?

**Major J A Manifold** Mr Chairman, I don't know whether it is the custom for Professor PLATT to wait until the discussion is finished or to answer questions at once, but if he is going to wait may I be allowed to steal some of his thunder. It was the last words of the previous speaker, Dr DAVEY, that saved his bacon. In the first part of his discourse he had dragged a "hormonal" red herring across the path of those of us who have recently been trying to deal with nutritional conditions in India and other parts of the world.

I have been in many tropical countries in the last 10 years and have been appalled at the lack of money and the consequent lack of protein in the diet which is so universal. Surely the whole thing can be summed up very easily in the fact that although money is the root of all evil, lack of it and lack of protein intake go hand in hand. I did not really understand the conditions existing in our Empire until I saw the awful poverty that exists in India, the "brightest jewel in our crown". We are about to lose her but if we rid ourselves of our responsibility for her we shall also lose our debt to the Indian races, which is to raise their standard of living. Our great handicap in dealing with chronic malnutrition rather than frank deficiency states has always been to lay our hands on some particular recordable point, and I would ask Professor PLATT whether the people working on nutrition and those working on blood could not possibly work more closely together? The only index of any value that I have found consistently associated with a chronic nutritional deficiency is the lower haemoglobin level, yet when one reads papers on malnutrition, the authors have usually passed very lightly over the haemoglobin level. Most of the statistics are built up on personal observation rather than on indisputable scientific fact, *e g*, one records this man has hair standing up straight, whether

it is curly or dry or glossy and whether he has a sore tongue a coloured tongue and so on. All such records are too liable to personal interpretation and variation, whereas by using the haemoglobin level, say per 100 of population, we have a recordable index based on a single scientific observation. At the present time there is considerable variation in the methods used to record haemoglobin levels, but I understand that the National Physical Laboratory in conjunction with Professor E. J. KING has produced what I believe will be an answer to the problem, i.e. a really useful standard haemoglobinometer using photometric principles.

With a low protein level due to chronic protein deficiency you usually get a low haemoglobin level and I feel that the method of HYNES and LEHMAN using a modified Van Slyke specific gravity method, enables us to survey an enormous number of people very quickly. It does not take long to take a drop of blood and blood films can be made at the same time. Surely malaria, poverty and low nutrition go hand in hand and I feel that if we concern ourselves with tangible results about the nutritional state of the Colonial Empire, we shall then almost automatically get rid of malaria.

I ask Professor PLATT whether a yardstick of nutrition could possibly be constructed which would be rather more scientific and not so liable to attack on the grounds of personal observation than those used at present. Could one possibly say that the haemoglobin level per 100 of population is so and so and therefore the state of nutrition is such and such?

Dr O. C. Chesterman: Mr President, if I say something in defence of the ignorant man, woman and a hoe it will not be because I want to detract from the urgency of the theme of tonight. It will only add to the urge we all feel that we are in some way responsible for the condition and must remedy it. My own experience has been with a good type of negro in the forest area where crops can be sown and harvested all the year round. There I have been impressed with the good physique of the people and their good food sense, whether this be instinct or tradition. The woman when pregnant is always given the best fish. Eggs and fowl are taboo to her but she gets her increased protein from the fish. She also adds to her diet a certain kind of earth which is rich in calcium. The natives know the need for accessory food factors. One favourite sauce is a mixture of salt, palm oil, lemon juice and red pepper. There you have A, C and D and any deficiency of B is made up from palm wine. Instinct or custom assures the accessory factors, and the exchange of products between fishing, hunting and agricultural tribes leads to a reasonable distribution of the nutritional factors required. The opposite is only too often the case when industrialization comes in, or when you have men crowded together in prisons and other places. Professor PLATT started his lecture with a diagram showing the weight of babies according to maternal

protein intake Native women seem to have realized this themselves His last remarks raised the question of birth control Here also native tribes have something to teach us The husband is often kept as a grass widower until the woman has finished suckling the last child There is something to be said for the ignorant man, woman, and the hoe if left reasonably free to look after themselves But having come in to upset his simple economy, it is obviously our duty to set him up on a higher level

**The President** If I understood Major MANIFOLD correctly, he rather suggested that a single test among the African people would be the haemoglobin test, and that if that was done it would show whether the subject was suffering from malnutrition, and it would be unnecessary to go into all the details of stiff hair and so on Is that so?

**Major Manifold** Not exactly What I really meant was that it was something absolutely recordable, an absolute scientific fact

**The President** That the low haemoglobin is an effect of malnutrition?

**Major Manifold** Yes

**Dr Lucy Wills** I don't feel competent to speak on the matter, but broadly I think I should agree that we could get some indication of malnutrition from the presence of anaemia For example, in my experience, if people were well fed the anaemia associated with hookworm infestation was never as severe as in ill-fed populations, and Europeans on their high protein diet hardly suffered at all from anaemia, even with heavy infections But though widespread anaemia in a population can be considered an index of malnutrition, a population may suffer from a deficiency state without very appreciable anaemia In individuals anaemia is no indication of their nutritional state

**Lieut-Colonel J H Walters** Mr President, I would like to quote some examples drawn from the Indian Army because, although this subject is not quite within the ambit of the discussion, one gets some idea how this malnutrition occurs There is a special Indian Army diet both for vegetarians and non-vegetarians Compared with the figures the Professor has given, the essentials are well above the minimum requirements The Sepoy gets about 3,500 calories a day provided he eats all his diet, yet you get the curious anomaly on active service in base areas of men showing grave nutritional deficiencies in the midst of plenty That can usually be explained as a deficiency in animal protein In the Indian Army we get quite a number of controlled experiments in that a single unit living under identical conditions, and exposed to the same



risks of infection, is divided into two types of man—the man who gets a small meat ration in addition to his basic vegetarian diet, and the man on a vegetarian diet alone. All of us who deal with Indian troops have had examples of grave malnutrition arising among the vegetarians while the meat-eating elements in the same unit are perfectly nourished. In Egypt, from a base area, from one such unit, I treated sixteen cases of grave malnutrition amongst the vegetarians and none at all from the meat-eating company. In a base hospital in India I recently treated forty two cases of grave nutritional deficiency from one battalion evacuated from Burma, of whom thirty-nine were drawn from the vegetarian Jat companies and only three from the meat-eating Rajputana Mahommedan companies, although the unit was composed of Jats and Mahommedans in the proportion of 2 : 1. The deficiency syndromes are, first of all, a grave macrocytic anaemia. A man with this is very weak, but he walks in somehow. He may have a haemoglobin of only 3.5 grammes per 100 c.c. The second manifestation is the sprue syndrome. Amongst such vegetarians one sees cases of very severe sprue with marasmus atrophy of the tongue and gross steatorrhoea. This, in my experience responds to heavy doses of nicotinic acid, although at the height of their diarrhoea they appear to be unable to absorb this substance through their alimentary canal. I have given such a man 1 000 milligrammes daily by mouth, yet he has failed to show the slightest symptoms of vaso-dilatation yet such cases do improve dramatically when they are given nicotinic acid parentally in doses of 150 to 200 milligrammes a day. This malnutrition is conditioned by prejudice and custom. The Jat in his village is a fine healthy fellow. I have caught civilian relatives of my Sepoy patients, and examined them as controls. They were extremely healthy people. The reason is that they dislike any form of animal protein other than milk, and in the Army on active service you can only give 2 ounces a day of condensed milk, which is quite insufficient. In Burma, after the campaign was over and in Malaya long after peace was proclaimed, you still got these heavy wastages because such men could not be persuaded to take a reasonable meat ration.

Dr H. S. STANNUS. From the discussion which has followed Professor PLATT's very interesting paper one lesson would appear to emerge—interference on our part with the native customs of the native populations of Africa is associated with a lowered state of nutrition. Endemic diseases and occasional famine may take their toll but on the whole the bulk of the population was

Since the meeting, Lieut.-Colonel O'DWYER, R.A.M.C., has brought to notice increases in the milk ration for vegetarian troops, which were brought into force on the Burma front late in 1944 with considerable reduction in the wastage of manpower through malnutrition. Yet many cases of grave deficiency disease from Malaya, treated by me in India during the past 6 months, stated that no such increased milk ration had reached them.—FD

healthy and happy Dr DAVEY's remarks were perhaps the most important contribution this evening and worthy of the gravest consideration. The whole problem of native nutrition had to be tackled as Professor PLATT has said, but the subject of nutrition is by no means yet fully understood, and it is to be hoped we shall proceed slowly and avoid the mistakes of the past.

**Professor B S Platt** (in reply) Obviously there is much more that has to be done before we really understand what is happening in tropical ulcer. Lesions such as these may be the result of factors from two or three groups—infection, stress factors of various kinds as well as malnutrition, there is also the time factor to be taken into account, also the results of such disturbance as liver damage over periods of years interfering with metabolism.

About the endocrine influences—no doubt there is a relationship between them and state of nutrition. The best example, perhaps, is that of iodine in nutrition in relation to the function of the thyroid gland. I remember an old African gentleman in a certain area in East Africa being very much worried because the young women of his tribe were maturing much earlier than they should have done to fit in with the time of the initiation ceremonies, and they attributed it to a change from the use in the diet of potashes to common salt. Some day we may discover a relation between potassium and sodium contents in the body and some of the factors controlling endocrines and the sex glands.

I agree with Dr WILLS about the place of haemoglobin levels as an index of the state of nutrition. We do take measurements of haemoglobin levels in nutrition surveys and they are of value. There is evidence that reduction in haemoglobin levels can contribute to the development of signs of insufficiency of certain nutrients. This is not surprising since most dietary substances are concerned in processes involving oxidation—and we depend on haemoglobin for the carriage of oxygen to the tissues of the body. It is incorrect to say that our observations are based on impressions, our records are based on physical signs of disease of the skin, mucous membrane and so on.

I have got no answer for Dr DAVEY. I do not know what is in the mind of the Secretary of State about the control of game. Dr CHESTERMAN was speaking about the ways of the African. I could add many more examples, and I have learned to be very respectful of traditional practices and old wives' tales in any country. Those who know some of the work we have done on the fermentation process in Kaffir beer\* will appreciate that we have been able to produce evidence that some of these products should not be discarded from native diets without putting something else of equal or better food value in their place. I am puzzled by the observation about the small amount of meat in the diet of Indian troops making all the difference to their state of nutrition,

\* PLATT, B S (1946) *Proc Nutr Soc*, 4, 2

it seems to me there must be other factors at work—perhaps some of long standing

Finally referring to Dr STANNUS's remarks, I prefer to use the word sophistication rather than "civilization" when applied to some of the changes which are taking place. I regard civilization as being characteristic of the Chinese scholar and his capacity for enjoyment of some of the simple joys of life, a cottage, good food and wine and literature. It is a rather different picture from that which one sees in, for example, the back streets of Lagos. The trouble is that changes are going on so fast that we cannot afford to wait. I think we have to act quickly. I think we have to avoid the mistakes we made in our own industrial revolution. We have the benefit of seeing the mistakes we made in our own country and we must not repeat them in Africa. We have got to get a move on but we must do the job properly when we do it.

## COMMUNICATIONS.

BY

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AND

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Early in the spring of 1943, in a small isolated station on the North-West Frontier of India, some men of an Indian infantry unit reported sick, complaining of sore throat, hoarseness and dysphagia. In a few cases there was aphonia. Routine clinical examination did not reveal any obvious cause for these symptoms apart from some congestion of the pharynx and soft palate, but the presence of a vitamin B<sub>2</sub> complex deficiency was immediately suggested by the presence of a characteristic glossitis associated in some cases with angular stomatitis. All men in the unit were examined, and out of 509 seen, 104 showed well developed mouth or throat lesions, distributed as follows —

	More than one sign	One sign only
Glossitis		17
Angular stomatitis		2
Injection of the soft palate		—
Pharyngitis		2
	<div> <div> <div>29</div> <div>}</div> <div>—</div> </div> <div> <div>19</div> <div>}</div> <div>—</div> </div> <div> <div>35</div> <div>}</div> <div>—</div> </div> </div>	

\*We wish to thank the DIRECTOR OF MEDICAL SERVICES in India, and the D M S, 11 Army Group, S E A C, for permission to publish this paper, and Brig F HARRIS, C B E, M C, for enabling us to make the investigations. We are also very grateful to numerous officers, in particular Lieut -Colonel J J O'DWYER, R A M C for valuable assistance and encouragement.

Seventy-two of these cases also showed changes resembling maceration of the mucous membrane of the cheeks, at the level of occlusion of the teeth. A few men complained of lachrymation, but no eye lesions were visible on ordinary examination.

Enquiries as to the general physical efficiency of the men in this unit as a whole indicated that though they were considered to be reasonably fit for war, staleness had been apparent for several weeks, manifesting itself as absence of high spirits and impaired capacity for sustained hard work. Though the men could still march long distances, they could not undertake much physical labour at the end of the march, as is normally expected in a really fit unit.

#### PRELIMINARY INVESTIGATION.

Some yeast autolyzate (vegemite) and nicotinic acid were immediately available, and so sixty men were chosen at random from the 104 cases already seen and divided into three groups of twenty each, for therapeutic tests.

*Group 1* Each man was given 500 mg. of nicotinic acid by mouth daily for 14 days. There was no improvement in the lesions, and in five cases the glossitis became worse.

*Group 2* Each man received 1 oz. of yeast extract daily. Improvement in the tongue condition was evident after 7 days and after 14 days all cases were either cured or much improved.

*Group 3* No treatment was given. There was increased severity of lesions in four cases, and in the others no change.

On completion of this preliminary trial, the whole unit was given 16 fluid ounces of milk per man daily in addition to the usual rations. A limited stock of yeast extract was also given to all the men (whether showing mouth lesions or not) in doses of  $\frac{1}{2}$  oz. daily for 7 days, followed by  $\frac{1}{4}$  oz. thrice weekly for 3 weeks. At the end of the month, of the original 104 well-developed cases, there were only eight who still showed definite lesions, and these were much improved. No fresh cases appeared during this time. The officer commanding the unit remarked on a new spirit of liveliness and energy apparent among his men.

The extra milk and yeast extract were then stopped. After 3 weeks on ordinary rations only sixty-three of the cured cases had relapsed and seventeen new cases had appeared.

Meanwhile, the following facts regarding the previous history of the unit emerged. It had arrived in British India from an Indian state in 1940 since when it had served in various stations in the North West Frontier region. The daily routine had as a rule been very energetic and during the previous year or so hard training had been a definite formation policy. In the autumn of 1942 there had been a very high incidence of malaria which necessitated giving a course of "blanket" quinine treatment to the whole unit. Since

that time, unit health had picked up, and the existing impairment was attributed by unit officers partly to the residual effects of malaria and partly to staleness resulting from strenuous training. It was evident, however, that this particular unit had not suffered from malaria to an exceptional degree, as compared with other units in the station, nor had its training programme been more strenuous. The rations in use had not changed much, and were not significantly different from those of other units in the same locality. Though no men from other units had so far reported sick with similar symptoms, it was decided to inspect all troops in the station. As a result, 312 cases of well-marked glossitis, etc., were found. The condition was appearing evenly among all races present in the station, which included Jats, Kashmiris, Nepalese, Madrassis and men from frontier tribes. Evidently a defect in the rations was affecting the whole station. (Subsequent investigation of the position in other stations throughout India brought to light other cases, but nowhere was there an epidemic of similar severity.)

#### MAIN INVESTIGATION

One hundred cases were selected for special study, on the basis of having a well-developed glossitis. For administrative reasons, they were taken from two units only, the battalion concerned in the earlier investigation, and another infantry unit of different racial composition. Detailed clinical examination of the cases preceded therapeutic tests on controlled dietary. All these men were carefully segregated and maintained on a diet identical with that issued in their units during the previous month, as ascertained by survey. Owing to the isolated position of the station, only minor laboratory studies could be made, and limited supplies of synthetic vitamins prevented therapeutic trials on a more comprehensive scale.

#### CLINICAL DESCRIPTION

The lesions found in this group of 100 cases were distributed as follows —

Glossitis	100
Glossitis with fissuring	44
Injection of the soft palate	85
Injection of the pharynx	72
Injection of the epiglottis	33
Rhinitis	12
Cheilosis	21
Angular stomatitis	23
Maceration of the buccal mucous membrane	39
"Vascularity" of the cornea	62
Proctitis	48

*Glossitis*—An early and a late stage were noted. In the early stage there was stripping of the fur and epithelium from the edges and tip of the tongue (most marked at the tip) leaving these areas smooth, with flattened papillae, and dark red or magenta in colour. The centre of the tongue was usually covered with thick white fur. In the second stage stripping was complete leaving a smooth but glossy tongue, usually somewhat swollen and showing lateral indentations caused by the teeth. The papillae were flattened and the whole tongue was dark red or magenta in colour. Of the 100 cases thirty-four had an early stage and sixty-six a late stage glossitis. In two-thirds of the latter the tongues were fissured. In three cases the fissures were longitudinal but in the others eight or more fissures, each about half an inch long ran transversely into the edges of the tongue. The fissures were deep and raw as if the edges of the tongue had been slashed with a knife. We cannot from personal experience compare this glossitis with that of pellagra, but the appearances were quite different from those we have seen in Indian patients suffering from chronic severe secondary malnutrition involving multiple vitamin B complex deficiencies, a condition having many features in common with pellagra. In the latter the tongue is usually small, glazed, with rather shallow longitudinal fissures and scarlet in colour.

*Pain* was remarkable by its absence considering the tongue appearances. Fifty-four men however complained on questioning of soreness and burning while eating spiced food.

*Angular stomatitis* was present in twenty-three cases, and was identical with that described in the literature as characteristic of riboflavin deficiency. In a few cases the fissures had become indurated and were painful.

*Cheilosis* was found in twenty-one cases. It was never severe and took the form of peeling of the central portion of the lips, leaving these areas glossy and bright red in colour.

*Lesions of the buccal mucous membrane* were present in all cases with angular stomatitis, and also in sixteen cases which did not show the latter. A streak of white macerated mucous membrane extended from the angles of the mouth backwards towards the anterior pillars of the fauces and lying parallel to the line of occlusion of the teeth. It resembled a white chalk line drawn across the inner surface of the cheek. In three cases superficial ulcers were present in the same situation.

*Injection of the soft palate* present in eighty-five cases, was limited strictly to the soft palate so that there was a sharp line of demarcation between the red or bright scarlet soft palate and the pale hard palate, at the posterior margin of the latter.

*Pharyngitis*—Congestion of the oro-pharynx was present in seventy-two cases.

*Larynx*—Forty-two men gave a history of recent hoarseness and dysphagia. In four cases there had also been aphonia. Laryngoscopy was

performed on the hundred men, and in thirty-three congestion of the epiglottis was observed

*Rhinitis*—Twelve cases showed crusts and scaling on the mucous membrane of the anterior nares, suggestive of subacute rhinitis (One case, not included in this selected group showed bilateral vertical fissuring of the mucocutaneous junction of the nares)

*Eyes*—None of these men complained voluntarily of eye symptoms, but on direct questioning twelve said that their eyes watered to an unusual degree. Ordinary examination revealed no lesions other than five cases of pterygium. There were no visual defects in the group, ninety-nine men reading 6/6 with each eye on Snellen's types, and one man 6/9 with each eye. In no case was there staining of the cornea with fluorescein. A slit lamp microscope could not be obtained, but examination with a lens showed that sixty-two men had one or more capillary twigs apparently crossing the limbus and invading the clear cornea. No arcades could be seen, and the appearances did not resemble those described by FERGUSON (1944).

*Proctitis* was present in forty-eight cases. The skin around the anus was dry, and even gentle tension on the buttocks was sufficient to cause slight fissuring and bleeding. The mucocutaneous junction at the anus was macerated and had a white pulpy appearance, exactly like that at the mouth in angular stomatitis. In more severe cases there was anal fissuring. Proctoscopic examination revealed a dry, dull, dark red mucous membrane which bled very easily. Those lesions were symptomless, apart from slight tenderness.

*Skin condition*—One case had a well marked, scurfy dermatitis of the scrotum. Otherwise, apart from two cases of acne vulgaris, there were no defined skin lesions in this series. All skins, however, were rather dull and rough. Seborrhoeic dermatitis about the nasolabial region was not present.

*Faeces*—There was no diarrhoea. Twenty-eight cases showed infestation with *Ancylostoma duodenale*, one with *Ascaris lumbricoides*, one with *Enterobius vermicularis*, and one with *Taenia saginata*. The finding of hookworm ova was not correlated either with the presence of a proctitis or with the general severity of other lesions.

*Blood*—A haematological examination limited to haemoglobin estimation (Sahli) and red cell counts revealed no significant abnormality.

*General physical condition and age*—Sixty-nine of the men were between 20 and 30 years of age; eleven were under 20, and nineteen were over 30 years old. All were of reasonably good physique, and on casual inspection appeared to be perfectly fit, by ordinary standards.

*Mental condition*—The men were somewhat depressed, apathetic and lethargic. The chattering laughing and singing usually heard in the barrack rooms of Indian troops was absent. They tired fairly easily, and, as one officer put it, did not seem to be "trying". This applied not only to the selected cases, but also to the units generally from which they had been drawn. It



is, however important to realize that from the unit officers point of view the situation was readily accounted for by staleness, and they saw no cause for alarm.

#### THERAPEUTIC TRIALS

One of the hundred cases was sent on furlough during this investigation, and is excluded. The remaining ninety-nine cases were divided into eight groups matched as far as possible for distribution and severity of the various lesions, and were treated as follows —

*Group I*—Six men, given 3 mg. of riboflavin daily by intramuscular injection for 7 to 14 days, depending on the rapidity with which the lesions healed.

*Group II*—Eight men, given 5 mg. of riboflavin daily by mouth for 10 to 14 days.

*Group III*—Nine men, given 1 oz. of yeast autolysate (vegemite) daily for 11 to 22 days, depending on the rate of response.

*Group IV*—Ten men, given 1 oz. of dried brewer's yeast daily for 10 to 22 days.

*Group V*—Ten men, given 1 c.c. of crude liver extract by intramuscular injection on alternate days for 18 days, each man thus getting a total of 9 c.c.

*Group VI*—Two cases were given 500 mg. and three cases 250 mg. of nicotinic acid daily by mouth for 14 days.

*Group VII*—Ten men, given 15 minims of halibut liver oil and 100 mg. of ascorbic acid for 15 days.

*Group VIII*—Forty-one men maintained on the controlled diet without any special treatment.

The results will be summarized. Among the men in Groups I and II, with one exception, all tongues healed rapidly (in 7 to 10 days, but up to 14 days in the case of fissured tongues). In one case that showed no change of the tongue condition (though the other lesions cleared up), fissuring was exceptionally deep, and the man stated that his tongue had been like that all his life. Angular stomatitis (three cases), cheilosis (two cases) and maceration of the buccal mucous membrane (six cases) healed in 6 to 10 days. Congestion of the soft palate, pharynx and epiglottis (present in thirteen, ten and six cases, respectively) either cleared up completely or nearly so. In the two men in these groups who showed rhinitis, the crusting and scaling disappeared after 5 days treatment. In ten men proctitis was present. This healed relatively slowly but in eight cases healing was complete after 14 days treatment and in the remaining two cases there was much improvement. The single case with scrotal dermatitis was placed in Group I, and this condition cleared up after 4 days treatment. There was no appreciable difference in response to parenteral and oral riboflavin therapy.

In Groups III and IV seventeen out of nineteen cases cleared up. In one

of the remaining two cases the glossitis improved greatly but did not entirely disappear. In the other case the glossitis did not change, though all other lesions improved. Improvement was generally slower than that obtained with riboflavin, and in no case was the proctitis affected. This is attributed to insufficient duration of treatment, since in the riboflavin-treated groups, proctitis healed more slowly than the other lesions. No regular change in corneal "vascularity," as observed with a lens, was evident among any of the cases in these or other groups.

Among these men, treated with riboflavin or yeast, a remarkable improvement in mental condition was evident, which was all the more noticeable by contrast with those in other groups, who remained listless and morose. A trial at the end of the course of treatment showed that it was easily possible for an uninitiated non medical officer to select from a mixed group a majority of those men who had received curative treatment, merely through their cheerful bright-eyed expressions and the lively interest they took in their surroundings.

Among men in Groups V to VIII, the lesions were either unaffected or became worse. The negative response to liver extract may have been due to insufficiently large dosage, or, possibly, to lack of potency of the preparation, which was not a well-known brand.

Sixty-three men gave a history of malaria during the previous 12 months, but there did not seem to be any relationship between the number of attacks and the severity of the deficiency signs. It was, however, possible to observe the immediate effect of malaria on the lesions, since seven cases developed the disease while under our observation. In two cases the lesions were unaffected, but in the remaining five definite and rapid deterioration occurred. Two men developed new angular stomatitis 4 days after the onset of fever, and in the other three smooth stripped tongues developed fissuring for the first time, after 3 to 5 days. The onset of fissuring was dramatic in its rapidity and extent.

On completion of the therapeutic trials on controlled dietary, all completely cured men were returned to their units, and the remainder, including all those in Groups V to VIII, were given 1 oz. of yeast extract daily for a month. At the end of this time, all lesions, except "vascularity" of the cornea, had disappeared completely. Again the improvement in mental condition was striking.

#### SUBSEQUENT HISTORY

Shortly after the completion of the above investigation, all units were placed on a new ration scale (described on p. 407). Two months later only fifty six definite cases of the deficiency state remained in the station. It seemed, therefore, that the new diet was exercising a curative effect. (This conclusion, however, must be qualified, since there was no control which would

has excluded the possibility of a seasonal variation. We have the impression, from general observation that this deficiency state among new recruits tends to be more common in early spring than at other times of the year. That the diet was, in fact, able to control the deficiency was confirmed by inspection of many other troops who have used it, and from observations on recruits who entered the Army showing similar lesions.

Ninety-seven men of the unit in which the condition had been first noted were re-inspected after they had been on the new diet for 14 months. Only two men showed evidence of glossitis which was of a very mild degree. No associated signs were observed. Both these men were considerably older than the average: indeed one was about to be discharged as too old for further service.

#### DIETARY BASIS OF THE OUTBREAK

In order to appreciate the prime causes of the outbreak—which is believed to be the only epidemic outbreak of primary deficiency disease among Indian troops during the 1939-45 war—it is necessary to sketch the administrative background. Before the war of 1914-18, Indian troops in India were fed according to a system whereby each man received a cash allowance with which he bought his own rations from the regimental contractor. There was no control whatever over the nutritive value of the rations consumed. Experience of widespread scurvy in Mesopotamia in 1916 (where rations supplied in kind were inadequate) resulted in a belated appreciation of the importance of nourishing troops well as opposed to merely satisfying their hunger and a new system of rationing in India was introduced. This consisted of issuing a number of relatively non-perishable foods in kind supplemented by a cash allowance, whereby perishable foods (including incidentally most of the protective foods) were bought locally in the open market. An important difference from the old cash system was that money was no longer given to individual soldiers but was spent on a unit basis by the commanding officer who was responsible for the health of his men and could obtain medical advice. The system worked satisfactorily during the peace. After 1939 market prices of foods began to rise, and successive increases in the cash allowance were made. In due course however medical officers were beginning to point out that in some places local supplies could scarcely meet the demand at any price and therefore the system could no longer be depended upon to provide troops with a theoretically adequate ration. Arrangements were therefore put in hand to supply complete rations in kind to all Indian troops according to a fixed scale of quantities. The outbreak of riboflavin deficiency described above occurred shortly before the revised scale was brought into use. Its appearance in one particular station can be attributed to the unusually isolated situation of the place in a barren countryside where fresh food were particularly scarce and expensive.

Clinical manifestations appeared first in one unit, probably because of a less careful control of expenditure of the ration money than that which had pertained among other units. Though there were no grounds for criticism on the score of unit administration at the time of the investigation, it was ascertained that some time previously the custom in this unit had been to give half the ration money to individual soldiers—a partial reversion to pre-1914 practice—so that there was nothing to prevent this sum being spent, for example, on cigarettes instead of food. At the time of our investigation, however, it was evident that an adequate diet could not be obtained from local markets even with the most careful budgeting. The following table shows the rations actually used by units at this time and the revised ration scale in kind which was subsequently put into force. The latter was a general measure throughout India, and was not devised specifically to deal with any outbreak of deficiency disease. It was subsequently replaced by a better scale.

	Rations which led to the outbreak	Revised ration scale
Rice (parboiled)	12 oz daily	12 oz daily
Atta (wheatmeal)	12	12
Dhall (pulses)	3	4½
Ghee (a)	2 "	2 "
Salt	½-1 "	½-1 "
Sugar	1½ "	2 "
Meat (b)	8-9 " weekly	2 "
Vegetables (c)	2-3 daily	10
Milk	1½-2 "	6 "
Tea	1½-2 " monthly	½ "
Spices	4½-7 "	½ "
Fruit	Nil	4 thrice weekly

- Notes (a) Ghee is clarified butter and was issued about 5 days in a week. "Vegetable ghee" (hydrogenated oil) was issued on the remaining days.  
 (b) Goat meat, containing about 50 per cent by weight of bone.  
 (c) About 50 per cent root varieties and 50 per cent green and other varieties.  
 (d) Issued in kind according to a fixed scale.  
 (e) Purchased locally by units with the ration money.

Disregarding cooking losses, the original diet provided (very approximately) 1.1 mg of riboflavin, and the revised diet about 1.4 mg.

It is of interest that the original diet resulted in clinical riboflavin deficiency only. As far as we could judge there was no evidence of incipient clinical scurvy nor of vitamin A deficiency (A high proportion of vitamin A in milk ghee is destroyed by the cooking methods normally employed). Our impression is that the minimum requirements for adults of vitamin A and ascorbic acid are considerably lower than is usually claimed. We were certainly unable to demonstrate any impairment of military efficiency directly attribut-

able to vitamin A or vitamin C deficiency notwithstanding the so-called biochemical lesions which must have been present.

### DISCUSSION

The occurrence of the syndrome in epidemic form and its rapid cure by synthetic riboflavin leaves little doubt that the men were suffering from primary riboflavin deficiency. This is confirmed by the composition of the diet. The *symptomatology* shows some minor differences from that described by JONES *et al.* (1944), which is believed to be the only previous description of a similar epidemic. We are unable to account for these differences, since the response to synthetic riboflavin was equally satisfactory in both outbreaks. An *ad hoc* explanation, such as on grounds of racial differences, is unsatisfactory.

The vascularity of the cornea, as observed with a lens, did not respond to any form of treatment. It was quite different from the biomicroscopic capillary invasion of the cornea described as typical of riboflavin deficiency by FARCUSON (1944) and may have been caused by continual exposure to sun glare and to dust rather than by a dietary deficiency.

The remarkable change in mental outlook produced by specific therapy indicates that mild riboflavin deficiency can be an important cause of impaired morale and physical efficiency among troops. Though JONES *et al.* do not stress the psychological aspects of the deficiency state, the following sentence from their paper is significant. 'A early as the second day (of treatment) they became full of thanks, which is the more remarkable because these people are usually grudging in their gratitude.'

There is evidence that fever as in malaria, can precipitate riboflavin deficiency among men who have subsisted on a borderline intake. The experience of the authors with Indian troops—apart from that recorded here—indicates that in a minority of afebrile individuals the characteristic lesions may persist even when the diet is apparently quite adequate. In such cases, a defect of absorption or metabolism probably exists. As a rule however a diet of the type described will clear up all major signs within 3 to 6 months. The process is accelerated by giving extra milk.

SWASINATHAN (1942) drew attention to the fact that although riboflavin deficiency must affect every cell in the body the lesion appears only at certain sites, and that no explanation for this distribution had been offered. More recently STANLEY (1944) suggested that the derangement of tissue function is relatively greatest in those tissues which possess the highest degree of capillarity and whose metabolism is greatest. We find it somewhat difficult to explain the detailed effects of the condition as seen by us, in terms of STANLEY'S hypothesis. It is suggested that a wear and tear mechanism is also operative as follows. The tongue lips, buccal mucous membrane, soft palate pharynx and epiglottis nose anus and lower rectum and scrotal skin (and, perhaps,

the eyes) all have one feature in common—the epithelium is exposed to a relatively greater degree of wear and tear than other epithelial surfaces. Under normal conditions, regeneration keeps pace with wear and tear. In riboflavin deficiency, interference with cell metabolism impedes epithelial regeneration, and failure to keep pace with destruction occurs first at those sites where wear and tear are greatest. Individual differences of wear and tear may, perhaps, explain variations in symptomatology from case to case.

Another cause for differences between cases or groups of cases is suggested by the possible functional inter-relationship between various members of the vitamin B complex, as outlined, for example, in SWAMINATHAN'S review. On this hypothesis, different deficiencies may give rise to a somewhat similar clinical picture and one member of the complex, given in sufficiently large doses, may in some cases be able to compensate at least in part for deficiencies of other related members. It is thought that some such mechanism is necessary to explain the rather conflicting opinions in the literature of riboflavin deficiency, and also the remarkably specific effect of synthetic riboflavin in the cases described in this paper. It is difficult to believe that a natural diet of the type consumed could give rise to uncomplicated riboflavin deficiency. In our cases, however, nicotinic acid intake seems to have been fully adequate, which is in accordance with the analysis of the diet.

#### SUMMARY

- 1 The occurrence of an epidemic condition among Indian troops responding to treatment with synthetic riboflavin or yeast is described
- 2 The dietetic origin of the outbreak is discussed
- 3 A possible explanation for the distribution of the lesions is suggested

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## OCULAR SYMPTOMS IN PRISONERS OF WAR IN SUMATRA

BY

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A good deal is now being heard of an ocular syndrome which is extremely widespread among released prisoners of war from the Far East and elsewhere. The syndrome, which causes a serious diminution of visual acuity, may occur in association with beriberi or pellagra or independently of these diseases. It may or may not be accompanied by various other neurological phenomena, such as ataxy, paraesthesiae and nerve deafness, or dermatological phenomena, such as angular stomatitis, glossitis, scrotal dermatitis, etc. The lesion is probably a retrobulbar optic neuritis due to a dietary deficiency most likely connected with the  $B_{12}$ -complex, but some prefer to call the condition an optic neuropathy until the nature of the lesion can be more definitely ascertained. Toxic and infective factors cannot be entirely excluded. The ocular signs are not always constant and yet a review of the now quite extensive literature since a similar condition was first mentioned by STRACHAN in *Sago's Annual* for 1888, and described by him in 1897 shows a uniform thread running through the whole, so that one can scarcely doubt a similar causation in the outbreaks described in various parts of the world.

An account of the symptomatology of the syndrome from notes made at the time of its occurrence may not be without interest. It must be stated that shortage of staff, absence of an ophthalmologist or any reference material, and the general conditions of captivity make the observations very incomplete. The cases here described occurred among 490 British Service men, prisoners of war of the Japanese at B Camp Palembang Sumatra. The men had been captured about the time of the fall of Singapore on 15th February 1942. Many complaints of visual disturbance were being made, so between 30th July and 10th August 1943, all men who complained of any recent ocular disorder were interrogated by the writer and this account refers to 126 such men. 26 per cent of the population of the camp at the time. The men had then been for some 18 months on a diet deficient in protein, fat and the B group of vitamins. The deficiencies were not however as gross as they became later, no deaths were occurring, beriberi was very rare—I can recall only one patient with



the ocular syndrome giving a clear history of recent beriberi—and pellagra did not make its appearance until the following year but other signs of malnutrition were prevalent. On 17th November 1944 a rough survey of all the 1,225 men then in camp was done for signs of malnutrition and 395 men (32.2 per cent.) complained of visual disorder. The diet was by then much worse but had not yet reached its lowest level, and it is worthy of note that ocular disorders were more conspicuous in 1943 than at a later date when beriberi and pellagra were common and men were dying daily of starvation.

The majority of the men examined in 1943 complained of a syndrome characterized by photophobia and blurred vision, both on reading and for distant objects often described as at a distance of 20 yards or more. There was also usually pain and infection of the cornea and watering of the eyes in the early stages. A few atypical cases are included.

#### DURATION OF OCULAR DISORDERS AT TIME OF EXAMINATION

	Cases.	per cent.
Under 1 month	9	7.1
1-3 months	20	15.9
3-6 months	49	38.9
6-12 months	29	23.0
Over 1 year	18	14.3
Indefinite	1	0.8
	<hr/>	
Total	126	

*Onset.* The onset was as a rule fairly gradual the visual defect taking weeks or months to develop fully but in ten cases (7.9 per cent.) the onset was stated to be sudden and two of these patients were unable to read within a week of the onset of symptoms.

*Progress.* At the time of observation cases were classified as follows:—

	Cases.	per cent.
Getting worse	17	13.5
Stationary	63	50.0
Improving	44	34.9
Cured	2	1.6
	<hr/>	
Total	126	

*Signs and symptoms.* The typical patient complained of gradual onset of photophobia with marked sensitiveness to the sun, some irritation and watering of the eyes, some pain in or around the eyes increased by exposure to the sun or in some cases by reading and blurring of vision. After a few weeks or months the irritation and pain, and sometimes the photophobia, ceased or decreased but the defective vision improved more slowly or persisted. At the

time of observation twenty-two patients (17.5 per cent) were for all practical purposes unable to read, and a further fourteen (11.1 per cent) could only read with great difficulty. The majority of these severe cases were still unable to read on release in 1945. Not one became blind. One of the most severe cases which I saw in Singapore after release from another camp could only tell the time by a kitchen alarm clock by holding it within a few inches of his eyes. The prognosis in such cases is likely to be poor, and MOORE (1937) states that cases who have had the condition for over a year usually fail to respond to treatment or are partially cured with residual optic nerve changes, but a few of my patients who had previously been unable to read had with little or no treatment recovered sufficiently to be able to do this at the time of observation.

Blurred vision was specifically complained of by eighty-eight patients (69.8 per cent) and only a few cases stated that vision was not affected. In many cases blurring of vision was stated to be worse for distant objects, over 20 yards, in some for near objects, but in few cases with blurred vision reading was not affected. Some patients said that on reading words became jumbled up or that they could only read by picking out each individual word slowly, some described difficulty in focusing for reading. In the great majority of cases the condition was bilateral, but in a few cases only one eye was affected or one more than the other. One patient complained of a blank area in the centre of his field of vision, another of blank eccentric areas. It was not possible to map fields of vision. Night blindness was not complained of.

I gained the impression that in a number of my cases the effects of an existing error of refraction were accentuated, and STRACHAN (1897) stated that a latent error of refraction unnoticed before the attack, might call for correction later, while RIDLEY (1945) observed that all his ex-prisoner cases were unduly presbyopic and RICH (1946) noted a high proportion of myopes among his cases.

Pain was complained of by seventy-one patients (56.3 per cent). It was very variable both in intensity and location. It occurred behind the eyes and across the forehead in a number of patients, in and around or above the eyes, in a few. One man complained of pain on opening and closing the eyes, and two of a pricking sensation in or behind the eyes. Pain on moving the eyes was not specifically complained of, but enquiry was not made for this symptom. Pain was frequently caused or accentuated by exposure to the sun, sometimes by reading. Pain as well as other symptoms was often not constant, occurring in attacks, and it often cleared up later in the course of the disease in patients with a residual visual defect.

Photophobia was an almost constant complaint and nearly all patients were unduly sensitive to sunlight but six (4.8 per cent) stated that they were definitely not sensitive to sunlight, and in a further seven (5.6 per cent) the condition was not noted. Irritation and injection of the cornea were noted in eighty-one cases (64.3 per cent) and in forty of these cases (31.7 per cent) it was

a major feature of the condition. In some cases it appeared only on exposure to the sun or on reading. One patient had for several months repeated sudden attacks of most acute and intense inflammation of the conjunctiva with much watering and photophobia. These attacks came and went with the most extraordinary rapidity usually lasting for a few hours. The condition eventually cleared up. Photophobia, like pain, was often less marked or absent in the later stages of the disease. The syndrome cannot have been caused by sun glare since seven patients (56 per cent.) were employed only in the camp, not on outside working parties and so were not unduly exposed to sunlight.

One patient complained of dryness of the eyes and a few that the lids were stuck together in the morning but there was no case of purulent conjunctivitis in this series. Watering of the eyes was sometimes profuse. There were two cases of frank iritis and one of corneal ulcer healed before the observations were made. One patient complained of dizziness. Later when food conditions were much worse a number of cases of a different syndrome were observed: these patients suffered from attack of true vertigo occurring in any position, and some showed nystagmus.

Pupil reflexes were unfortunately observed only in a few cases and in these they were normal. Ophthalmoscopy was done in a few cases in the early stages and nothing abnormal was noted. Several months later ophthalmoscopy was done on a few cases with severe residual visual defect and temporal pallor of the discs, sometimes very marked was a constant feature. This sign is noted by many writers.

#### ACCOMPANYING SIGNS OF MALNUTRITION

The main accompanying signs of malnutrition were as follows —

	Cases	per cent.
Angular stomatitis	57	45.2
Glossitis	48	38.1
Angular stomatitis and glossitis	29	23.0
Generalized stomatitis	2	1.6
Cheilosis	5	4.0
Neuritis, paraesthesiae	32	25.4

The appearance of these signs of malnutrition, particularly the dermatological signs, was noticeably intermittent and they were not present in all patients at the time of examination. The incidence of these conditions among the men suffering from the ocular syndrome was almost certainly considerably higher than among the remainder of the prisoners, but I cannot prove this by figures.

The word neuritis is used to cover a number of somewhat vague paraesthesiae including burning feet, pains in the legs and numbness and tingling of the extremities and sometimes of the lips. Burning feet, a very

widespread sign of malnutrition among prisoners of war in the Far East, was previously well known among Indian estate labourers in Malaya and curiously enough was often associated with nostalgia occurring almost exclusively among those who had recently arrived from India. It was one of the earliest signs of malnutrition to appear in our camp, the first two patients being officers in whom the nocturnal pain was so severe as seriously to affect their mental condition. The condition is mentioned by SCOTT (1918) and LANDOR and PALLISTER (1935) and it was also noted in the Spanish Civil War.

One patient slowly became almost completely deaf with no sign of otitis. Apart from this case no nerve deafness was observed but it is possible that minor degrees may have passed unnoticed.

An outbreak of scrotal dermatitis swept the camp in May 1943 but it was of a more acute type than one would expect to be caused by malnutrition. The red sore and weeping scrotums suggested an infective origin; the majority of cases cleared up within a fortnight and only a handful showed a tendency to become chronic. The men were grossly overcrowded, clothing was quite inadequate and washing facilities bad while the camp was infested with bed bugs. During long experience in Malaya I have never seen anything like this condition before nevertheless the scrotal condition did in many cases resemble that described and illustrated by LANDOR and PALLISTER (1935) occurring among Asiatic prisoners in Malaya; it was the most troublesome lesion of a syndrome ascribed by them to vitaminosis B. I feel therefore that a nutritional factor cannot be excluded in our cases.

The general physical condition of the patients suffering from the ocular syndrome was roughly classified as follows:—

	Cases	per cent
Good	63	50.0
Fair	42	33.3
Poor	15	11.9
Not noted	6	4.8
Total	126	

It was very noticeable that some of the patients with the worst visual defect were in good physical condition and the incidence of the syndrome could not be correlated in any way with the physical condition. Many of these severe cases remained in comparatively good condition when the diet of the camp reached a very low level and beriberi and pellagra were prevalent.

#### Treatment

Treatment was entirely inadequate and the results inconclusive. Green bean *Phaseolus aureus* Roxb. (*P. radiatus* Linn.) was given to some patients when it could be obtained, also marmite but the quantities were inadequate for a fair

test. Vitamin A-D preparations were used both internally and as eye drops. Atropine drops gave some relief in the more severe cases. Dark glasses were provided whenever possible. One patient was given twenty-one tablets of lactoflavin, 1 mg. and reported some improvement; no more could be obtained. Two men volunteered the information that the condition improved when they were able to obtain an increased amount of vegetables.

### REVIEW OF LITERATURE

STRACHAN (1897) saw many hundreds of cases of a form of multiple neuritis prevalent in the West Indies. The condition was characterized by paraesthesiae, dimness of vision, defective hearing and excoriations with some desquamation at the edges of the lids and margins of lips and nostrils. Burning sensations in palms and soles were accompanied by heat perceptible to the touch of the examiner. The main nerves of the extremities, especially the ulnar, were sensitive to pressure and herpetic vesicles sometimes appeared along the course of the terminal filaments. There was a retrobulbar neuritis associated with scotomata. Wasting of muscles, monoplegias and facial palsies, ataxia and corneal ulcers were sometimes noted. Sensation was sometimes blunted but not abolished. A few cases were fatal from involvement of the heart and diaphragm, and autopsy showed conspicuous pigmentation of organs, especially the nervous system as well as the palms and soles. Liver and spleen were found to be as in malaria. STRACHAN considered that the condition was probably due to the poison of malaria. He considered and rejected the poison of beriberi as a cause. It is interesting to note that he recommended treatment by "nourishing food increasing in quantity and variety."

SCOTT (1918) described a similar but more acute condition, which he called central neuritis among Jamaican sugar cane workers on a very inadequate diet. There were a number of deaths and at autopsy the optic nerves showed widespread degeneration distributed along practically all the fibres. Masses of fat granules were found in the large nerve cell of the optic chiasma. Scott considered that the disease was an intoxication and excluded beriberi and pellagra as causes.

ELIJAH (1920) describes early Japanese work on the ocular sign of beriberi and states that KONO (1895) described retrobulbar neuritis with amblyopia, central scotoma and contraction of the visual fields but no fundus changes while other Japanese writers described partial atrophy of the temporal portion of the disc or the whole disc. The green bean *azuki kishiang hidjan* of the Netherlands East Indies *Phacelurus* Roxb. is prescribed for treatment by the Japanese.

MOORE (1930, 1931, 1932, 1933, 1934, 1937) has described a similar condition prevalent in Nigeria which rendered many natives nearly blind and was curable in the early stages by marmite or yeast. MILLER (1934) discussing

MOORE's work ascribes the retrobulbar neuritis to vitamin A deficiency. WRIGHT (1928, 1930) describes an A and B avitaminosis of Sierra Leone which often affected pregnant women. The signs were angular stomatitis with glazing of the skin at the canthi, glossitis, dimness of vision with conjunctivitis and lachrymation, paraesthesiae, ataxy and sometimes complete paralysis of the limbs. DAVIES (1927) described a syndrome met with in the African Hospital, Lagos, paralysis and nerve signs were conspicuous, and there was acute pharyngitis, often with glossitis, angular stomatitis, painful excoriations of scrotum and axillae, and retro orbital pain, dimness of vision and inability to focus. N A B was used in treatment.

LANDOR and PALLISTER (1935) describe a syndrome occurring among long-term Asiatic prisoners in Malaya, which closely resembles that described here. They attribute it to avitaminosis B<sub>2</sub>.

FUCHS (1936) describes retrobulbar neuritis as the chief eye complication of beriberi and states that it resembles the amblyopia due to abuse of alcohol and tobacco. He describes slight temporal pallor of the discs and draws attention to the different manifestations of beriberi in Japan where retrobulbar neuritis is common, and in Indo-China where it is rare, he points out that the disease runs different courses in different countries and states that the treatment of the retrobulbar neuritis is identical with that of beriberi. METIVIER (1941) discusses eye diseases due to vitamin deficiency in Trinidad and describes a syndrome similar to the present one.

ADOLPH *et al* (1944) and WHITACRE (1944) described conditions experienced by Americans in Japanese internment camps and mention dimness of vision as one of the signs of malnutrition. STANNUS (1944) gives a comprehensive review of the manifestations of hyporiboflavinosis and claims that the main effect is upon the capillary endothelium. He points out that many of the signs of hyporiboflavinosis may or may not be associated with true pellagra.

WILKINSON and AU KING (1944) describe an amblyopia due to an avitaminosis, occurring in Hongkong at a time when pellagra had made its first appearance in the Colony and had become a scourge. The condition was rare and they were able to collect only fifteen cases, of which only one was actually a pellagrin. Since the condition was painless they prefer to call it an amblyopia rather than a retrobulbar neuritis but temporal pallor of the discs was seen in three cases.

CHURCHILL (1945) discusses nearly 500 cases of deficiency disease among prisoners of war in Singapore and Thailand, and he records the diet scales which showed a deficiency of calories, vitamin B<sub>1</sub> and the B<sub>2</sub> complex. The ocular syndrome was very similar to that met with in Sumatra, but the incidence was much lower. SPILLANE and SCOTT (1945) describe retrobulbar neuritis sometimes associated with deafness ataxy and paraesthesiae occurring among German prisoners of war in the Middle East who had previously been exposed to dietetic deprivations which had caused an outbreak of pellagra.

Full vitamin treatment of SPILLANZ and SCOTT's cases gave inconclusive results. RIDLEY (1945) describes a high incidence of visual disorders among released prisoners of war and internees from Thailand. Practically all the released prisoners showed some kerato-conjunctival abnormality. RIDLEY attributes the ocular lesions to general malnutrition rather than to a simple deficiency of the B group of vitamins. Massive vitamin treatment gave some improvement in about half the patients in a fortnight. RIDLEY states that arrangement has been made to follow up all cases, British, Australian and Dutch. DANSEY, BROWNING and RACI (1946) discuss ocular signs among released prisoners of war at Rangoon who had had beriberi. Ten cases out of thirty had retrobulbar neuritis. GARLAND (1946) considers that the ocular sign which he saw in released prisoners from Rangoon, Singapore, Hongkong and Java were not due to B deficiency. He doubts whether B is a factor but states that the deficiency is probably in the B group of vitamins, though a deficiency of vitamin A has not yet been excluded. GOLDSMITH (1946) considers that the retrobulbar neuritis, which he has seen both among native troops in Freetown from 1941 to 1943 and among released prisoners of war from Thailand and Indo-China is probably due to a protein deficiency or rather to a poisoning by certain kinds of low-grade protein. His report uniformly negative results from vitamin treatment.

### CONCLUSION

An ocular syndrome has been described which affected one quarter of the undernourished prisoners of war living under bad conditions in Sumatra. The syndrome was accompanied in many cases by nerve and skin signs indicative of malnutrition, but it occurred apart from beriberi or pellagra.

Many questions remain to be answered about the ocular syndrome now found to be so widely prevalent among released prisoners of war. What relation does it bear to beriberi and pellagra, to the ocular syndromes described in the earlier literature and to the skin and nerve disorders so frequently but inconstantly described in association with them? One cannot escape the impression that there is some relationship. Does the disturbance in the visual pathway originate in the retina or in the optic nerve? The condition is sometimes described as an optic neuropathy until it has been established whether it is an amblyopia or a retrobulbar neuritis. Many cases occurred among Australian and sections of the brain complete from the eye to the occipital cortex have been taken to Australia and when description of these have been published the point may be cleared up.

The cause of the condition lies between a toxin, an infection and a dietary deficiency or as seems not improbable a combination of two or more of these factors. So far as my own cases are concerned the usual toxic causes of amblyopia, retrobulbar neuritis or ocular palsies can readily be excluded these include ethanol and fermentation product, benzene, trichlorethylene

lead, quinine, arsenic, iodoform, digitalis, etc.\* Certain species of *Dioscorea* which may be toxic cannot entirely be excluded as yams were issued in the rations and several cases of acute poisoning by "gadong," the tubers of *Dioscorea hispida* Dennst., occurred on one occasion. The cyanogen containing foodstuffs such as cassava should also be considered.

The possibility of a bacterial or virus infective factor must be considered. The incidence of the syndrome in many camps, a large number of cases occurring within a limited time, suggests this possibility. Optic neuritis may occur in the course of herpes ophthalmicus, mumps, chickenpox and other general virus diseases. It would be interesting to know whether the herpetic vesicles mentioned by STRACHAN have been observed by others. Absorption of toxins from infective skin disease is another possible factor. There was an appalling incidence of septic skin disease of all kinds in our camp. Palembang seems to be famous for this even in peace time, and I would attribute it to the general conditions of captivity rather than to a specific vitamin deficiency. There was no clear correlation between skin disease and the ocular syndrome. There was little evidence of vitamin A deficiency in the camp only one or possibly two mild cases of follicular keratosis or phrynoderma being seen. At that time the men probably got enough vegetables and fruit to supply the minimum, if not the optimum, quantity of vitamin A and C needed.

If dietary deficiency plays an important part in the causation of the syndrome, is the deficiency single or multiple, of protein or of vitamins, and if of vitamins, what is the relative importance of lack of A, B, the known B<sub>2</sub> complex, or some hitherto unidentified factor? Data will later be available from Singapore and Hongkong camps correlating the incidence of the ocular syndrome with the food intake. My impression is that my own cases were probably examples of hyporiboflavinosis with a possible added infective factor, the lesion being a retrobulbar neuritis.

The evidence as to prognosis is extremely conflicting, and it will be most interesting to hear to what extent patients with residual visual defect will recover.

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\* Tobacco can be excluded since fourteen (11.1 per cent) of my patients were non-smokers.



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THE COMPLEMENT FIXATION REACTION IN ASIATIC  
SCHISTOSOMIASIS EMPLOYING CERCARIAL ANTIGEN  
(*SCHISTOSOMA SPINDALE*)

BY

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The group nature of the complement fixation reaction in bilharzial diseases of mammals has been emphasized by the work of FAIRLEY, CAWSTON, MINNING and other observers. FAIRLEY (1919) showed that a cercarial antigen of *Schistosoma mansoni* would detect antibodies produced by both *S. mansoni* and *S. haematobium* in man, and alternatively that a similar cercarial extract of *S. haematobium* was an efficient antigen in the serological diagnosis of infestation with either or both of the Egyptian schistosomes.

CAWSTON (1921) in South Africa recorded that an alcoholic extract of the livers of *Physopsis africana* infected with the cercariae of *S. bovis* acted as an efficient antigen for the serological diagnosis of *S. haematobium*, and *S. japonicum* and *S. bovis* in cattle.

\* Thanks are due to Brigadier N. HAMILTON FAIRLEY, CBE, FRCS, for his interest and helpful criticism and to Squadron Leader W. P. HARVEY DAKIN and Flight Lieutenant J. CONNELLAN for their co-operation during this investigation.

In India FAIRLEY (1936) found that cercarial extracts of *S. spandale* were equally efficacious in detecting infection by *S. haematobium* and *S. mansoni* in man or *S. indicum* and *S. spandale* in goats. Later in England FAIRLEY (1933) found that the sera of goats experimentally infected by Prof. R. T. LIEPKE with *S. m. illicet* or *S. bovis* yielded positive reactions when tested against cercarial antigen derived from *S. spandale*.

The complement fixation reaction with cercarial antigen (*S. spandale*) has thus been successful in detecting infestations with three human and four cattle schistosomes and it is group applicable to mammalian schistosomes established.

MIXWINE (1941) in Germany found complement fixation and skin tests of considerable value in diagnosis of isolated cases of bilharziasis and of infestations which had existed for a long time in which eggs could not be found. Precipitation reaction were of no value. MIXWINE used alcoholic extracts of the digestive glands of *Planorbis guadelupensis* (*P. glabratus*) infested with *S. mansoni*. These reacted not only with sera from patients infested with *S. mansoni* but also with sera from patients infested with *S. haematobium* and *S. japonicum*. Both FAIRLEY and MIXWINE recorded that extracts of non-infested snails liver gave negative reactions with bilharzia-positive sera while FAIRLEY (1930) also found that livers of snails infected with non-bilharzia cercariae (*C. bombayensis*) failed to deviate complement in the presence of bilharzia-positive sera.

The above data indicate that the complement fixation test with bilharzia cercarial antigen is specific for the genus *Schistosoma*.

During 1927 and 1928 Lieut.-Col. F. P. MACKIE, of the Haflikine Institute, Bombay despatched to the Walter and Eliza Hall Institute (a) 1 gramme of dried snails livers (*P. exustus*) infected with cercariae (*S. spandale*) sealed in a glass ampoule and (b) 40 c.c. of cercarial antigen in sealed ampoules, each containing 1 c.c.

This antigen has been prepared in India by FAIRLEY's technique in the following manner —

Snails of the species *P. exustus* which were ejecting cercariae of *S. spandale* were dissected and the livers or digestive glands separated. Each organ was examined microscopically and if heavily infected it was teased out with needles and placed in a vessel, absolute alcohol being added in the proportion of 1 c.c. to each liver. After shaking for 20 minutes the preparation was extracted for 24 hours at 37° C. It was then thoroughly shaken again and was run through filter paper. The clear yellow filtrate was concentrated in a water bath at 45° C. by bubbling air through the solution with an exhaust pump until it became turbid. Just enough alcohol was then added to clarify the solution and the concentrated extract was put up in 1 c.c. ampoules and stored in the

ice chest until required. In the text this antigen will be referred to as Antigen B.

During the intervening years Antigen B has, from time to time, been used at the Hall Institute, Melbourne in complement fixation tests for the diagnosis of schistosomiasis in patients from other countries who were known to have been exposed to infection with *S. haematobium* and/or *S. mansoni*. Some of these patients had received no treatment, and in these ova were usually present in the urine (*S. haematobium*) or faeces (*S. mansoni*). Others had already received full courses of treatment, and although ova could no longer be demonstrated, the sera from certain of these individuals continued to give positive reactions, in some instances up to as much as 30 minimum haemolytic doses (M.H.D.) of complement being fixed.

These findings indicated that the antigen had retained its potency, and that it, therefore, could be used in the investigations described in this paper.

## I—PRESENT INVESTIGATION

This report is concerned with the results of serological tests carried out on 560 members of the R.A.A.F. who had been exposed to bilharzia infection over a period of 16 days at Leyte, an endemic focus of Asiatic schistosomiasis in the Philippines.

During this time, most of the men swam in the Bislig River which was later found by FAUST and his colleagues to harbour snails (*Schistosomophora hydrobiopsis*) infected with cercariae of *S. japonicum*. Details of this outbreak of Asiatic schistosomiasis as it affected R.A.A.F. personnel are to be recorded elsewhere by DAKIN and CONNELLAN and, therefore, need not be further discussed in this paper.

## II—TECHNIQUE

The technique used was similar to that fully described by FAIRLEY (1919) and FAIRLEY and WILLIAMS (1927) and FAIRLEY, MACKIE and JASUDASAN (1930). For economy in reagents standard dropping pipettes were used. The unit of volume was 0.1 c.c. (2 drops) and the total final amount in each tube was 0.5 c.c. (5 volumes).

Antigen B standardized in the usual way was diluted 1/40 with normal saline and titrated in detail in the presence of complement to detect any anti-complementary activity.

**Complement**—Pooled complement was obtained from the sera of healthy male guinea-pigs and the M.H.D. of complement determined by accurate titration each day. In the main test 3, 4½ and 6 M.H.D. of complement were used.



TABLE I

COMPLEMENT FIXATION TESTS AND STOOL EXAMINATION OF 560 R.A.A.F. PERSONNEL  
POSSIBLY EXPOSED TO INFECTION WITH *S. japonicum*

Number of sera tested	M H D of complement deviated	Complement fixation		Stools	
		Positive	Negative	Ova found	No ova found
				(Positive)	(Negative)
391	0	0	391	5	386
22	3	22	—	12	10
25	4	25	—	18	7
10	5	10	—	10	—
11	6	11	—	6	5
11	7	11	—	8	3
9	8	9	—	9	—
9	9	9	—	9	—
11	10	11	—	10	1
9	12	9	—	8	1
12	15	12	—	11	1
6	18	6	—	6	—
19	20	19	—	18	1
2	25	2	—	1	1
5	30	5	—	5	—
1	35	1	—	1	—
7	40	7	—	7	—
560		169 (=30.2%)	391 (=69.8%)	144 (=25.7%)	416 (=74.3%)

the sera were positive and ova not demonstrable. As many of these individuals had already received treatment, some 6 to 8 months previously, the relationship of treatment to the positive and negative findings in the series are summarized in Table II (p. 426).

This series of 560 cases includes 195 men who had, some 6 to 8 months before these tests were done, been given treatment with either foudadin, tartar emetic, or tartar emetic and foudadin. No complement fixation tests had been done at the time. In a small proportion of these men, ova had been found in the stools prior to treatment. The remainder of the group were treated on the basis of a suggestive history and symptomatology, and/or arbitrarily, because of an eosinophilia of 10 per cent. or more. In view of the presence of eosinophilia in certain R.A.A.F. personnel not exposed to bilharzia infection it is probable that a number of individuals treated exclusively for this reason may not have been suffering from schistosomiasis.

TABLE II  
RELATION OF TREATMENT TO POSITIVE AND NEGATIVE FINDINGS.

	Treated.	Not treated
Positiv. C.F.T.	179	
Positive stools	113	66
Positive C.F.T.	30	
Negative stools	22	8
Negative C.F.T.	5	
Positiv. stools	4	1
Negative C.F.T.	186	
Negative stools	84	102
	Total 500	
	193	307

(The results obtained by complement fixation and by stool examination after the initial course of treatment are shown in Table III.)

Since 60 per cent. of these men subsequently showed ova in their stools, it is obvious that the treatment adopted had not been generally effective. It is not possible to deduce from the available data whether had the infestation been eradicated the complement fixation test would have become negative with the passage of time.

The remaining 331 were originally regarded as not infected and were not treated at the time (Table IV). On re-examination of blood and stools during the investigation, 7.39 per cent. showed ova in their stool and 9.3 per cent. gave positive complement fixation tests. Thus in 90.7 per cent. of cases originally regarded as non-infected, confirmation of this opinion was obtained by a detailed laboratory investigation undertaken 7 to 8 months later.

#### COMPARISON OF THE COMPLEMENT FIXING POWER OF SERA WITH THE OVA CONTENT OF THE STOOL.

In the whole series there were seventy-nine cases (twenty untreated, fifty nine treated) in which the sera fixed from 3 to 7 M.H.D.s of complement. In these, ova were present in the stools in 68.3 ( $\pm 0.2$ ) per cent. In a further ninety cases fourteen untreated seventy-six treated, in which more than M.H.D.

TABLE III  
ANALYSIS OF 195 TREATED CASES

Number of sera tested	M H D of complement deviated	Complement fixation		Stools		Treatment		
		Positive	Negative	Positive	Negative	Fouadin	Tartar emetic	Tartar emetic and Fouadin
				Ova found	No ova found			
60	0	0	60	4	56	58	2	0
18	3	18	0	10	8	14	3	1
14	4	14	0	11	3	12	0	2
8	5	8	0	8	0	8	0	0
10	6	10	0	6	4	7	2	1
9	7	9	0	7	2	9	1	0
8	8	8	0	8	0	6	2	0
6	9	6	0	6	0	6	0	0
9	10	9	0	8	1	9	0	0
7	12	7	0	6	1	5	0	2
11	15	11	0	10	1	10	0	1
6	18	6	0	6	0	6	0	0
17	20	17	0	16	1	13	2	2
2	25	2	0	1	1	2	0	0
4	30	4	0	4	0	4	0	0
	35	0	0	0	0	0	0	0
6	40	6	0	6	0	4	0	2
Total 195		135	60	117	78	172	12	11
		60.2%	30.8%	60%	40%	88.2%	6.2%	5.6%

were fixed, the corresponding percentage is  $94.4 (\pm 2.4)$  per cent. This difference of 26 per cent between the two groups is highly significant, as the possibility of it occurring by chance is less than 1 in 1,000.

Among the fifty-nine treated cases fixing 3 to 7 M.H.D.s of complement ova were found in stools in  $71.2 (\pm 5.9)$  per cent and in the seventy-six treated cases fixing more than 7 M.H.D.s ova were found in  $93.4 (\pm 2.8)$  per cent. Here again the possibility of this difference being due solely to chance is less than 1 in 1,000. It would therefore appear that a strong positive complement fixation test is almost certain evidence of existing infection. A weaker reaction (fixation of less than 7 M.H.D.) may in some cases indicate only the presence of residual antibody from a previously treated infection.

In thirty-five cases primarily regarded as negative and not treated one gave a negative serological finding but ova were found later in the stools twenty



TABLE IV

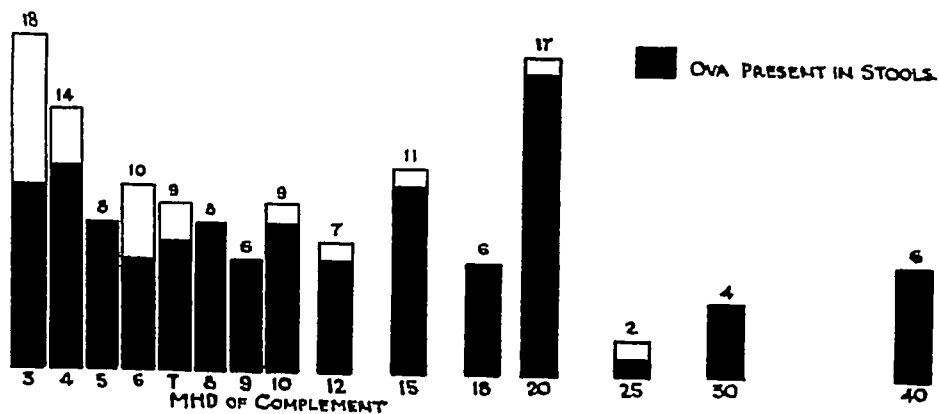
ANALYSIS OF 345 CASES PREVIOUSLY REGARDED AS NEGATIVE AND NOT TREATED.

Number of sera tested.	M.H.D. of comple- ment.	Complement fixation.		Stools.	
		Positiv.	Negative.	Ova found.	No ova found.
				(Positiv.)	(Negativ.)
231	0	0	231	1	230
4	3	4	—	2	2
11	4	11	—	7	4
2	5	2	—	2	0
1	6	1	—	0	1
2	7	2	—	1	1
1	8	1	—	1	0
3	9	3	—	3	0
2	10	2	—	2	0
2	12	2	—	2	0
1	15	1	—	1	0
0	16	0	—	0	0
2	20	2	—	2	0
0	25	0	—	0	0
1	30	1	—	1	0
1	25	1	—	1	0
1	40	1	—	1	0
345		34 (-9.3%)	231 (-67.0%)	27 (-7.8%)	238 (-69.2%)

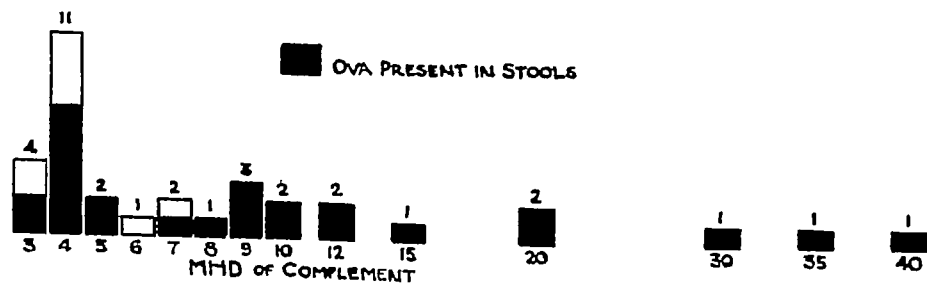
fixed from 3 to 7 M.H.D.s of complement and in 60.0 ( $\pm 11.0$ ) per cent. of these, ova were found in the stools; in fourteen, which fixed more than 7 M.H.D. of complement, all stools contained ova. Once more the possibility of this difference occurring by chance is very remote. (See Analysis on p. 429)

It should be noted, however that while the demonstration of ova in the stools is related to the presence of female schistosomes, antigenic stimulus may be provided by either males or females. Thus in goats experimentally infected with *S. spindale* and treated with emetine or tartar emetic, FAIRLEY and JASUDASON (1926) found that in certain animals only male schistosomes survived or that the ratio of male to female schistosomes was greatly increased (20:1). Female schistosomes appear more sensitive to treatment with these drugs and they suggested that the survival of male schistosomes following treatment afforded one possible explanation for the persisting positive reactions found in treated animals showing no ova in the faeces.

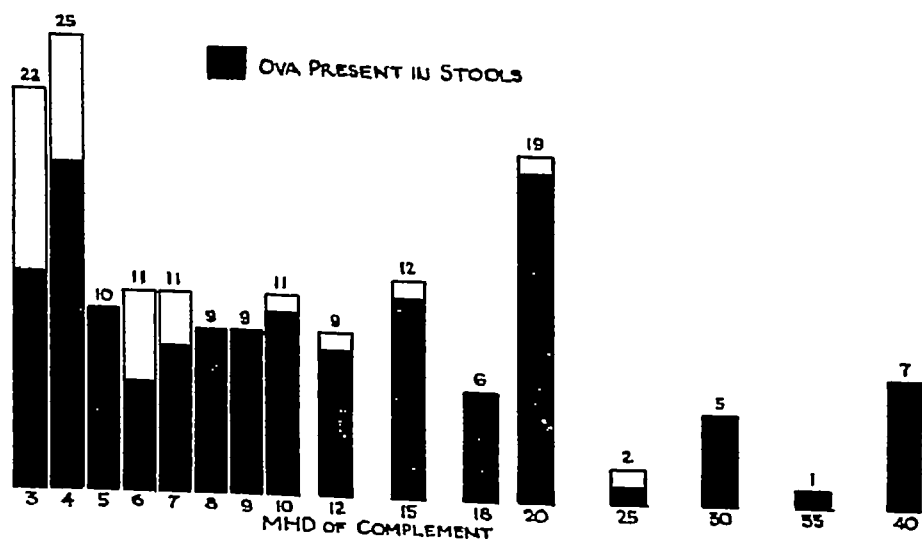
Later FAIRLEY (1933) recorded a series of patients in which strongly positive complement fixation reactions were observed to persist for 4 years or longer



ANALYSIS OF 135 TREATED CASES WHICH GAVE POSITIVE COMPLEMENT FIXATION



ANALYSIS OF 34 CASES, PRIMARILY REGARDED AS NEGATIVE AND NOT TREATED, WHICH GAVE POSITIVE COMPLEMENT FIXATION



ANALYSIS OF 169 CASES WHICH GAVE POSITIVE COMPLEMENT FIXATION





In Table VII it will be seen that when these antigens are used in a dilution of 1:40 in the presence of *Bilharziopsis acra*, there is more complement fixed with Antigen B than with Antigen D.L., but when the latter is used in a dilution of 1:30 there is no difference in the amount of complement fixed.

Alcoholic extracts made from freshly dissected snails' livers infested with *S. spindale* sealed in ampoules will keep for an indefinite period. Dried cercarial liers sealed in glass ampoules can also be kept indefinitely the alcohol extracts being made as required.

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## SICKLE-CELL ANAEMIA IN WEST AFRICA

BY  
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AND  
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Since the war renewed interest has been taken in sickle-cell disease or sicklaemia as it is met with in Africa (EVANS, 1944 and 1945, TROWELL, 1945, FINDLAY, ROBERTSON and ZACHARIAS, 1946, and BEET, 1946). The present communication deals with some of the many manifestations of the disease as seen in West Africa in soldiers and in African women attending hospital for a variety of diseases

### THE INCIDENCE OF SICKLE-CELL ANAEMIA

In America it has been estimated that among negro children in hospital the ratio of those having sickle-cell anaemia to those with the sickle-cell trait is 1 9. Among 500 children dying under 1 year of age and examined post-mortem by SMITH (1943) in Lagos, there were thirty cases of sicklaemia, of whom six were considered to have died from an acute sickle-cell crisis, a ratio between those with sickle-cell anaemia and the sickle-cell trait of 1 4. Among fifty-three persons of all ages showing sickling examined by us, ten were either having an acute haemolytic crisis or had a history of such crises, combined with a count of below 3,000,000 red blood corpuscles per c mm. Among 1,300 deaths of African soldiers occurring in the West African command there were twelve where an acute sickling crisis was regarded as the primary cause of death, while thirty-six other soldiers who recovered were admitted to hospital with acute sickle-cell anaemia. In thirteen patients suffering from other diseases an acute sickling crisis was regarded as a contributory cause of death. During the same period approximately 75,000 African soldiers were treated in hospital if 12 4 per cent of these exhibited sickling (FINDLAY *et al*, 1946).

\* Our thanks are due to Brigadier J B A WIGMORE, D D M S, West African Command, at the time these investigations were carried out. We owe a great debt to Dr A H CHENARD, Dr STANLEY COOP, Mr J S MCGREGOR and Dr R D REID of the Colonial Medical Service for their generosity in placing cases at our disposal, and to many officers of the Royal Army Medical Corps for information and assistance. Miss DOROTHY BARNFULL has given us much willing help.

the total number with sicklaemia would have been 9,300 and the ratio of those with acute sickling crises to those with sicklaemia would be approximately 1:151.

### FATAL HAEMOLYTIC CRISES.

Particulars of the twelve fatal cases of sickle-cell anaemia are shown in Table I. The age at the time of the fatal crisis varied from approximately 18 to 30 years with an average of just over 25 years. This bears out the finding in America that those with sickle-cell anaemia rarely survive beyond the third decade. In this series the fall in the number of red cells was the most obvious finding, the lowest red cell count on admission being 990,000, the highest 3,750,000 per c.mm. with an average count of 2,035,000 per c.mm. for the

TABLE I.  
FATAL SICKLING CRISES IN AFRICAN SOLDIERS.

Age	Temperature on admission, F	Red cells per c.mm. on admission.	Hb. % on admission.	Jaundice.
27	104.0	2,870,000	35	+
23	98	2,800,000	53	—
25	103.2	3,750,000	70	+
24	100.2	1,800,000	30	++
20	104.2	2,500,000	66	++
30	99.0	1,170,000	28	+
27	103.2	1,850,000	30	+
18	97.6	2,450,000	55	+
22	102.0	1,200,000	30	—
27	99.4	990,000	28	—
28	102.0	1,200,000	12	++
30	100.0	1,480,000	29	—

series. At the same time the haemoglobin percentage was low, the lowest being 12, the highest 70 per cent. with an average of 39 per cent. All patients had bilirubinaemia and eight of the twelve had definite jaundice of the conjunctivae. In all there was sickling of the blood *in vivo* as shown by the examination of blood taken from a vein under paraffin and fixed directly in formal saline under a liquid paraffin seal.

The following is an example of an acute sickling crisis in an African soldier —

Pts. E. A. S. Ashanti. Age 25 years. Service, 2 years. No significant previous history. Admitted 2.2.44 with temperature 103.2° F. morbilliform rash on face, trunk and arms, no lachrymation, no catarrh or cough, some generalized enlargement of all superficial lymph nodes. Diagnosed as dengue.





bacilli in sputum albumin in urine but no urobilin and no jaundice. Red blood corpuscles, 1,600,000 per c.mm. Hb. 28 per cent.; packed cell volume 17 per cent.; nine nucleated red cells per 100 leucocytes. Reticulocytes, 1 per cent. Red cells showed to some sickling with anisocytosis and poikilocytosis many target cells present. Bone marrow hypoplastic with very few early red cell precursors and only scanty normoblasts. Total leucocytes, 2,300 per c.mm. differential leucocyte count, polymorphonuclear leucocytes, 74 per cent.; lymphocytes, 21 per cent. mononuclears, 5 per cent. During the next 3 days the temperature varied from 98.4 to 101 F. Despite blood transfusion, death occurred after 3 days. The spleen, though normal in size, had large infarct at the posterior extremity both lungs showed diffuse tuberculous bronchopneumonia.

### ULCERS AND SICKLE-CELL ANAEMIA.

A relationship between sickle-cell anaemia and ulcers of the legs has been stressed in America by HERRICK (1910). In American negroes it is stated that ulcers occur at some time or another in 40 per cent. of those with sickle-cell anaemia. FINDLAY ROBERTSON and ZACHARIAS (1946) in West Africa could not detect any correlation between the incidence of ulcers and the general occurrence of sicklaemia. In the twenty five cases where a sickling crisis had been either a primary or secondary cause of death there were three with active tropical ulcers throughout the whole command the admission rate for tropical ulcers was thirty-eight per 1,000 admissions to hospital. Though the figures are too small for statistical purposes, they thus favour the view that active ulcers are more likely to occur in those with sickle-cell anaemia than in the general population. If old scars on the legs are also included, then thirteen of the twenty five cases showed evidence of having had ulcers on the legs at some time in their history a rate of 520 per 1,000. However among 1,073 African soldiers examined at random, 434 or 403 per 1,000, had old scars on their shins. It is of interest that ulcerative gingivitis was present in very marked degree in three of the twenty five fatal cases. Ulcerative gingivitis was a rare cause of admission to hospital in West Africa, the rate per 1,000 admissions for Africans being 1.05.

### HAEMOGLOBINURIA AND SICKLING CRISIS.

While bilirubinaemia and haemoglobinuria occur during a sickling crisis haemoglobinuria is of rarer occurrence. Theoretically if sufficient haemoglobin is liberated from haemolysed sickled cells there is no reason why the kidney threshold for haemoglobin should not be overcome. EVANS (1945) has described one such case we have now observed eight other cases in African soldiers and three in African women in which haemoglobinuria has occurred in association with acute haemolysis while sickling of the red cells has been demonstrated *in vivo* and has continued so long as the haemoglobinuria persisted.

It is obvious that before a diagnosis of haemoglobinuria due to a sickle-cell crisis can be sustained, other causes of haemolysis must be excluded. Three cases of paroxysmal haemoglobinuria, giving positive Donath Landsteiner reactions have been seen in West African soldiers but no case of acholuric

jaundice, march haemoglobinuria or of the Marchiafava-Micheli syndrome has been observed. Blackwater fever has occurred with increasing frequency in African soldiers (FINDLAY and MARKSON, 1946) and, since sickling can be demonstrated in the blood of 12.4 per cent of the African population (FINDLAY *et al*, 1946), it is obvious that some Africans who suffer from blackwater fever will also have the sickling trait. If sickling has no relationship to the haemoglobinuria there should merely be the delayed onset of sickling *in vitro* and no appearance of *in vivo* sickling. It may of course be argued that the onset of blackwater fever in an African liable to suffer from sickle cell crises is sufficient to precipitate one of these crises.

Particulars of the eleven cases where haemoglobinuria was associated with *in vivo* sickling of the red blood corpuscles are shown in Table II.

TABLE II  
HAEMOGLOBINURIA ASSOCIATED WITH *in vivo* SICKLING OF THE RED BLOOD CELLS

Sex	Age	Previous history of haemoglobinuria	Duration of haemoglobinuria in days	Result
M	24	+	2	Died
M	25	—	4	Recovered
M	22	—	12	
M	25	—	2	
F	16	+	2	
F	23	—	4	"
F	10	—	7	"
M	19	—	3	Died
M	23	—	5	
M	22	+	2	Recovered
M	23	+	5	

History of a previous attack of haemoglobinuria must in Africans be received with caution since any occasion on which the urine has been dark in colour may be regarded as due to the presence of blood pigments. In addition, at least 25 per cent of the male population has suffered or is suffering from bilharziasis.

The following are examples of haemoglobinuria associated with sickling *in vivo* and anaemia —

Pte E M, aged 25 years. Army service, 1 year 7 months. Pneumonia 6 years ago otherwise no complaints. Admitted to hospital 7.4.44, has felt unwell for 4 days, yesterday complained of acute epigastric pain and vomited all food. On examination temperature was 102° F, pulse 100, respirations 30. Liver and spleen not palpable, extreme tenderness in epigastrium. Icterus of conjunctivae. Urine contains albumin and urobilin but no haemoglobin, dark amber in colour. No malarial parasites seen in blood, sickling of red cells *in vivo*. Kahn negative.

8.4.44 Urine, very dark red in colour contains albumin, haemoglobin and urobilin, no casts but a few pus cells. Spleen not palpable but great tenderness under the left costal margin. Red blood corpuscles, 2,500,000 per c.mm. Haemoglobin percentage, 53 total leucocytes, 19,000 per mm. Sickling of red cells in vac. 300 c.c. of whole blood were transfused.

9.4.44 Haemoglobin percentage, 50 Sickling in vitro still present; haemoglobin present in urine.

10.4.44 Haemoglobin percentage, 60 only a few red cells show in vitro sickling urine free from blood pigment very faint jaundice of conjunctivae

14.4.44 Haemoglobin percentage, 72 no in vitro sickling

3.5.44 Haemoglobin percentage, 95 Complete recovery

Signalman S.B. Age 4 years. Army service, 1 year. Has had numerous attacks of malaria 3 months ago had haemoglobinuria and was in hospital for what was regarded as blackwater fever (no sickling tests performed). His father died 8 years ago from what is said to have been blackwater fever. For 3 weeks before admission he had felt very tired, and had aching pains in the bones with anorexia. 15.11.43 Severe aching in legs and arms 17.11.43 Vomiting 1 interval, stools very dark black in colour 18.11.43 Admitted to hospital complains of fever and aching in the limbs conjunctivae jaundiced. Temperature 100 F, liver 2 inches below the costal margin tender Spleen tender but not palpable. Urine contains albumin, urobilin and haemoglobin very dark red in colour. A rigor before onset of haemoglobinuria. No malarial parasites seen in the blood. Total red blood corpuscles, 1,180,000 per c.mm. Haemoglobin, 40 per cent.; reticulocytes, 4.8 per cent. total leucocytes, 10,400 per mm. Polymorphonuclear leucocytes, 50 per cent. lymphocytes, 42 per cent., large mononuclears, per cent. eosinophils, 6 per cent. Blood urea, 87 mg. per 100 ml. Kahn negative. Red cells show in vitro sickling 19.11.43 Haemoglobinuria continues granular casts and epithelial cells in the urine. Total red blood corpuscles 1,020,000 per c.mm. Haemoglobin, 35 per cent. packed red cell volume, 9.7 per cent. Sickling in vitro present. A packed red cell transfusion was begun, 2 pints being given in 13 hours with two rigors, temperatures up to 105.8° F. 20.11.43 Red blood corpuscles, 1,080,000 per c.mm. Haemoglobin, 33 per cent.; reticulocytes, 4.6 per cent. A few red cells show in vitro sickling. After 0300 hours no further haemoglobinuria was detected. The blood changes were as follows—

1943.	Hb percentage.	R.b.c per c.mm.	Reticulocytes percentage.	Blood urea mg. per 100 ml.
Nov. 18	40	2,180,000	4.8	87
19	25	1,020,000	—	—
20	33	2,088,000	4.6	—
(after transfusion.)				
21	42	2,220,000	5.3	—
22	36	1,600,000	5.3	64
23	36	1,910,000	—	—
24	40	2,000,000	—	—
25	43	2,200,000	5.2	40
26	49	2,420,000	5.0	—
Dec. 9	57	2,600,000	—	—

On 17.2.44 patient had a rigor and shortly afterwards passed dark coloured urine. On admission to hospital he was jaundiced and complained of pain and tenderness over the liver and spleen the former was 2 inches below the right costal margin, the latter just palpable. Red blood corpuscles, 3,000,000 per c.mm. Haemoglobin, 4 per cent. Blood cells showed in vitro sickling urine contained albumin, urobilin and haemoglobin. 18.2.44 Red blood corpuscles 2,500,000 per mm. Haemoglobin 33 per cent. Red blood

corpuscles still show sickling *in vivo*. Blood transfusion, 2 pints (temperature up to 105.0° F) 19.2.44. Red blood corpuscles, 2,500,000 per c mm. Haemoglobin, 38 per cent. Blood urea, 300 mg per ml. Icterus index 24. Van den Bergh immediate direct positive reaction. Plasma spectroscopically shows methaemalbumin. Only 5 ounces urine passed in 24 hours. Donath-Landsteiner reaction negative. A further transfusion was followed by a rigor (temperature 107° F) and death. Postmortem. Lungs oedematous and congested, pericardial cavity, 2 ounces bile-stained fluid, a patch of fibrinous pericarditis on the anterior aspect of the left ventricle. Liver very pale, spleen soft and friable, slightly enlarged with much perisplenitis. Bladder contained 2 ounces of blood-stained urine. Histologically, all organs were very congested, the capillaries being filled with sickled red cells showing commencing thrombus formation.

Ellen A. Age 23 years, married, no children. No periods for 3 months, had been anaemic since childhood and had occasional attacks of severe pain in the larger joints. Admitted 22.4.44 with a history of bronchitis for 1 week. Temperature 99.4° F. She complained of severe pains in the ankles and knee joints. Her conjunctivae were jaundiced. The liver was not palpable but the spleen was 1 inch below the left costal margin. The urine contained albumin, bilirubin and haemoglobin. The blood showed no malarial parasites but the red cells sickled *in vivo*. There was marked polychromasia with nucleated red cells, and many hypo- and hyper-chromic macrocytes. Haemoglobinuria and *in vivo* sickling continued for 4 days. No malarial parasites were seen in the blood. The blood changes may be summarized as follows —

Date 1944	Red blood corpuscles per c mm	Hb	Total leucocytes per c mm	Reticu- locytes per cent	Sickling	
					<i>in vivo</i>	<i>in vitro</i>
Apr 22	1,950,000	32	8,000		+++	+++
28	1,690,000	27	10,000	8	+++	+++
May 1	2,100,000	35	9,800	—	+++	+++
9	2,410,000	45	—	4	+++	+++
22	2,680,000	50	8,200	4	—	++
Oct 28	3,200,000	63	—	—	—	++

In these cases occurring in African females the symptoms, apart from *in vivo* sickling, are similar to those of the rare acute haemolytic anaemias of pregnancy seen in European women where there is jaundice, a reticulocytosis and, in severe cases, haemoglobinuria. WITTS (1932) considers that this anaemia of pregnancy is a true Lederer's anaemia.

#### PREGNANCY AND THE SICKLING CRISIS

REID (1936) showed that an acute sickling crisis may be associated with death during the later months of pregnancy. SODEMAN and BURCH (1937) found that in those with sickle-cell anaemia pregnancy aggravated the anaemia while KOBAC *et al* (1941) observed that women who suffered from sickle-cell anaemia frequently aborted. Our observations confirm these findings.

Of eighty pregnant women whose blood sickled *in vitro* on admission to hospital, twenty-eight died. Three of those who died produced dead babies at full time while four others who died aborted at from 3½ to 7 months. Most

commonly the pregnancy ends in the later months but in one patient three consecutive pregnancies had ended before the 4th month within a period of 2 years.

In order to throw light on the question of sickling and anaemia during pregnancy in Africa haematological examinations were made of 193 women divided into four groups. The average results are shown in Table III the seventy five European women who were not pregnant had been in West Africa for from 6 to 18 months.

TABLE III.  
HAEMATOLOGICAL DATA IN EUROPEAN AND AFRICAN WOMEN.

Group.	No. of cases.	Red blood corpuscles per c.mm.	Hb. %	C.I.	Leucocytes per c.mm.	Differential count.	
						Poly morpho-nuclears.	Lymphocytes.
(1) European women in Africa non-pregnant.	75	4,410,000	93.0	1.03	6,250	84.7	33.8
(2) African women, non pregnant, non-sickling.	47	3,074,000	63.8	1.03	4,800	65.8	22.6
(3) African women, pregnant non-sickling.	37	2,760,000	56.1	1.07	8,280	60.6	33.3
(4) African women, pregnant sickling.	34	2,210,000	43.8	0.84	8,180	55.7	27.5

It will be seen that in non pregnant African women the number of red cells and the haemoglobin percentage are both lower than in European women in pregnant African women the figures are considerably lower. RUSSELL (1911) has already drawn attention to the high degree of macrocytic anaemia in pregnant women but only nine out of the 100 women in her series were examined for sickling. The figures here recorded show that in pregnant women whose blood sickles *in vitro* the degree of macrocytic anaemia is more pronounced than in those pregnant women who do not sickle.

Acute haemolytic attacks may begin early in pregnancy and continue at intervals till a miscarriage occurs as the following case shows —

A.A., aged 20 years, when 18 weeks pregnant was admitted to hospital with haematuria, diagnosed as papilloma of bladder but on cystoscopy no papilloma and no evidence of schistosomes were found. Urine contained leucocytes and red blood corpuscles:

Kahn reaction negative Temperature 96 to 99° F, no jaundice Two years previously she had had a threatened miscarriage at 3 months and had finally miscarried at 4½ months After a week in hospital the haematuria had disappeared and she left hospital

When 26 weeks pregnant she was readmitted with severe headache, sharp abdominal pains and pains in the larger joints, no malaria parasites in the blood The blood showed immediate and complete sickling *in vivo* and *in vitro* She was breathless and had oedema of the feet and ankles Temperature 100.8° F The blood picture was as follows —

Red blood corpuscles, 1,200,000 per c mm Hb, 32 per cent C I, 1.33 Total leucocytes, 16,000 per c mm, polymorphonuclear leucocytes, 80 per cent, lymphocytes, 16 per cent, mononuclears, 2 per cent, degenerate forms, 2 per cent She was transfused with 500 c c of whole blood and given liver intramuscularly together with iron and left hospital after 9 days

Twenty-eight days later she was readmitted with acute abdominal pain and excruciating pains in the knees, ankles, and shoulders Six days later she miscarried, at the 30th week of pregnancy, the foetus was dead The patient had no complications, but when seen 2 months later she still had severe joint pains

The blood picture was then as follows —

Red blood corpuscles, 2,600,000 per c mm Hb, 48 per cent, reticulocytes, 5 per cent Total leucocytes, 12,000 per c mm She refused further treatment

There is evidence that the children of mothers with sickle-cell anaemia may die in a few days after birth, even when the baby itself is not apparently a sickler

The following cases illustrate this point —

M B, aged 25 years, anaemia. Blood sickled immediately and completely *in vivo* and *in vitro* Caesarean section performed at 34th week of pregnancy The child, a female, did not sickle but died on the 3rd day after delivery The only other child had died 2 years before, within a few days of birth

L T, aged 23 years, sickled immediately *in vivo* and *in vitro* Red blood corpuscles, 2,100,000 per c mm., Hb, 45 per cent Total leucocytes, 14,000 per c mm, P C V, 34 Full-time delivery, baby son sickled and died a few hours after birth with a ruptured spleen, no malaria parasites present in the foetal or maternal blood

E T, aged 30 years, jaundiced, complete and immediate sickling *in vivo* and *in vitro* Hb, 60 per cent, baby son did not sickle but died a few hours after birth from a spontaneous rupture of the spleen

W W, aged 16 years, sickled immediately, spontaneous delivery at 8½ months after five ante-partum fits, baby son did not sickle but died on 2nd day with an intracranial haemorrhage in a second pregnancy miscarriage occurred at 6½ months after a period of severe pains in the back and joints The blood picture was —

Red blood corpuscles, 1,600,000 per c mm Hb, 22 per cent, total leucocytes, 14,000 per c mm, differential count, polymorphonuclear leucocytes, 80 per cent, lymphocytes, 19 per cent, mononuclears, 1 per cent, reticulocytes, 8.2 per cent

As a rule patients with sickle-cell anaemia and haemolytic crises do not live beyond the third decade but as the following case shows sickle-cell anaemia may occur after the reproductive life is over

A P, aged 54 years, sickled completely in 12 hours, she had an attack of jaundice 11 years ago since when she has never been well During all this time she had been very anaemic, another attack of jaundice occurred 1 year ago She complained of indigestion and had such severe pains in the ankles, knees and shoulders that she was regarded as suffering from osteoarthritis On admission to hospital she was giddy, breathless and

commonly the pregnancy ends in the later months but in one patient three consecutive pregnancies had ended before the 4th month within a period of 2 years.

In order to throw light on the question of sickling and anaemia during pregnancy in Africa, haematological examinations were made of 193 women divided into four groups. The average results are shown in Table III the seventy five European women who were not pregnant had been in West Africa for from 6 to 18 months.

TABLE III.  
HAEMATOLOGICAL DATA IN EUROPEAN AND AFRICAN WOMEN.

Group.	No. of cases.	Red blood corpuscles per c.mm.	Hb. %	C.I.	Differential count.		
					Leuco-cytes per c.mm.	Poly morpho-nuclears.	Lymphocytes.
(1) E. women in Africa; non-pregnant.	75	4,419,000	93.6	1.05	8,300	80.7	35.8
(2) African women, non-pregnant, non-sickling.	47	3,078,000	83.8	1.03	8,600	68.9	32.6
(3) African women, pregnant, non-sickling.	37	3,760,000	86.3	1.07	8,399	80.8	31.1
(4) African women, pregnant, sickling.	34	3,210,000	83.8	0.94	8,380	81.7	37.8

It will be seen that in non-pregnant African women the number of red cells and the haemoglobin percentage are both lower than in European women. In pregnant African women the figures are considerably lower. RUSSELL (1941) has already drawn attention to the high degree of macrocytic anaemia in pregnant women but only nine out of the 100 women in her series were examined for sickling. The figures here recorded show that in pregnant women whose blood sickles *in vitro* the degree of macrocytic anaemia is more pronounced than in those pregnant women who do not sickle.

Acute haemolytic attacks may begin early in pregnancy and continue at intervals till a miscarriage occurs as the following case shows —

A.A., aged 20 years, when 18 weeks pregnant was admitted to hospital with haematuria, diagnosed as papilloma of bladder but on cystoscopy no papilloma and no evidence of schistosomiasis were found. Urine contained leucocytes and red blood corpuscles.

apparently good health, said he felt very hot and, after washing himself, collapsed and died. The spleen was 160 grammes in weight with red and friable pulp, the kidneys were swollen with numerous small haemorrhages in the cortex. The red blood cells had all undergone sickling. No other cause of death was found.

### DISCUSSION

The observations here recorded show that sickle-cell anaemia and acute sickle-cell crises are factors of considerable importance in the pathology of the West African native.

The symptoms described in West Africans are similar to those found in negroes in the New World. Joint pains and pains simulating an acute abdomen are not uncommon, presumably they are due to small thromboses and infarcts.

Small thromboses may be the cause of the liability to ulcers of the leg, first noted by HERRICK (1910) in association with sickle-cell anaemia, the underlying bone changes have also been suggested as a cause. It is of interest that while ulcers of the legs are not correlated with pernicious anaemia, they do occur in association with acholuric jaundice which has many similarities to sickle-cell anaemia. In two women with sickle-cell anaemia seen shortly after they had passed through haemolytic crises, severe gingivitis with Vincent's organisms also was present. These two patients both had purpuric spots with platelet counts of 120,000 and 300,000 per c mm.

Bronchitis was a not uncommon symptom, while intercurrent infections were frequent. Nervous symptoms were also noted. In one soldier who died with acute sickling and a small fibrotic spleen 2 in by 1.5 in, there was a cavernous sinus thrombosis, but this was associated with pneumococcal meningitis. In two other soldiers there was cerebral thrombosis with necrosis of brain tissue. In a boy, J. A., aged 10 years, who sickled completely in 24 hours, there was a thrombosis of the portal vein and enlargement of the spleen with much perisplenitis. Twelve weeks before being operated on he had had very severe left-sided abdominal pain lasting about a week. Despite the fact that his haemoglobin was 39 per cent and his red blood corpuscles 2,840,000 per c mm, he made a successful recovery.

At postmortem infarcts of the spleen have been found with considerable frequency.

A symptom which has only been previously recorded by EVANS (1945) in association with sickling crises is haemoglobinuria. In addition to the eleven cases here described, Dr R. D. REID informs us of a negro patient who had haemoglobinuria during an attack of yellow fever from which he died. At the postmortem the red cells were completely sickled and the spleen had the characteristic microscopic appearances seen in patients dying from sickle-cell anaemia.

So protean are the symptoms of sickle-cell disease in Africans that it is



collapsed; no jaundice but bilirubinaemia. Temperature, 100.2° F. The heart was dilated with haemic murmurs in all areas. The spleen and liver were not palpable or tender. The urine contained albumin.

The blood picture was as follows:—

Red blood corpuscles, 1,200 000 per c.mm. Hb., 16 per cent. Total leucocytes, 4,300 per c.mm.

She died 48 hours after admission and, unfortunately, no postmortem was permitted.

Although certain features of the case such as the absence of a leucocytosis and of immediate sickling suggest that this was not a typical crisis, the history of past crises associated with jaundice and profound anaemia and sickling suggests sickle-cell anaemia.

### THE DIAGNOSIS OF SICKLE-CELL ANAEMIA.

The cases which have been cited show that while the sickling trait is unassociated with any definite symptoms the sickle-cell crisis presents a very clear clinical picture of severe anaemia, weakness, bilirubinaemia, jaundice and possibly haemoglobinuria combined with severe pains in the larger joints and not infrequently pains in the abdomen or chest, together as a rule with fever. The presence of sickling *in vivo* or of immediate sickling *in vitro* confirms the diagnosis.

The symptoms associated with sickle-cell anaemia, apart from crises, are less easy to differentiate: thus they may be confused with tertiary syphilis, gonorrhoeal rheumatism, bilharziasis, anaemia due to malnutrition, malaria and helminthic infections.

In tertiary syphilis the pain is more marked in the shafts of the long bones than in the joints: in gonorrhoeal rheumatism and in septic arthritis due to staphylococci or streptococci the joints are swollen and inflamed.

In early bilharziasis there may be severe pains in the muscles, especially of the back, shoulder and chest: the joints are usually unaffected. Malaria may also give rise to aching in the muscles. In Africa many of these diseases may be found associated with sickle-cell anaemia in the same individual. From 25 to 50 per cent. of all troops in West Africa, for instance, suffer from bilharziasis. In the same way it is not uncommon to find that sickle-cell anaemia is combined either with macrocytic or dimorphic anaemia.

In sickle-cell anaemia sudden pain in the chest may stimulate coronary thrombosis.

Pte. F. aged 28 years, gave history of vague pains for many years. His haemoglobin was 52 per cent., total red blood corpuscles 3,250 000 per mm. His red cells sickled completely in 24 hours. During the course of pyrexial attack of unknown origin he suddenly developed acute praecordial pain shooting down the left arm. His temperature fell to subnormal and he was very collapsed. Electrocardiographic examination revealed no abnormality but X-ray of the chest showed an irregular mottling in the upper lobe of the left lung.

In unexplained causes of sudden death in Africans acute sickling appears to be a possible contributory factor. Thus L. Cpl. M.G. who had been in

apparently good health, said he felt very hot and, after washing himself, collapsed and died. The spleen was 160 grammes in weight with red and friable pulp, the kidneys were swollen with numerous small haemorrhages in the cortex. The red blood cells had all undergone sickling. No other cause of death was found.

### DISCUSSION

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So protean are the symptoms of sickle-cell disease in Africans that it is

a good rule to perform tests for *in vitro* and *in vivo* sickling in all cases showing anaemia and in all others where the diagnosis is in any doubt.

### CONCLUSIONS.

Sickle-cell haemolytic crises may occur in both sexes and at all ages in West Africans.

During the latent phase of sickle-cell anaemia there are no symptoms beyond occasional joint pains—a crisis is ushered in by joint pains, pains in the abdomen, over the region of the spleen, and sometimes in the chest.

The crisis is characterized by a great reduction in the number of erythrocytes by the presence of normoblasts in the peripheral blood, by a reticulocyte reaction and a leucocytosis, and by bilirubinaemia, if not by actual jaundice.

In a certain number of cases haemoglobinuria occurs, thus simulating blackwater fever.

Pregnancy predisposes to a crisis which generally though not invariably leads to death of the foetus. The infants of women who have passed through a crisis appear liable to sudden death within a few days of birth.

Blood transfusions are the only sure method of saving life during a haemolytic crisis.

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## THE VALUE OF PLASMOQUINE AS A GAMETOCIDE IN SUB TERTIAN MALARIA

BY

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The fact that plasmoquine has a destructive action on the gametocytes of *Plasmodium falciparum* has been known for a considerable time. There does not, however, appear to be any record of the duration of the life of crescents in the peripheral blood after treatment with quinine and atabrin. Nor is there any record of the effect of plasmoquine on the life of the crescent following such treatment.

These series of observations were carried out by us completely independently of one another in British Somaliland on unselected Somalis suffering from malaria during the months of February-May, 1944. One of us (R V B) working in the Mandera Area was dealing with adult enlisted Somali soldiers, and the other (G W A D) with Somalis of all age groups living in an isolated village in the Nogal Valley.

The incidence of gametocytes in Somalis suffering from sub-tertian malaria is relatively high, and crescents were found in 49 per cent of 215 consecutive adult hospital admissions suffering from malaria. Another set of observations showed that in 110 cases of malaria covering all age groups, 59 (54 per cent) at some time showed crescents in their peripheral blood. In both series of observations diagnosis was made by examination of thick films using Field's technique.

### TREATMENT

All cases of sub-tertian malaria were put on the following course of treatment as soon as the diagnosis of malaria was established microscopically. For the first 3 days quinine sulphate 10 grains t.i.d. in a mixture (quinine sulphate

\* We wish to thank Brigadier E R CULLINAN, Consultant Physician, E A Command, for his advice and suggestions in this series of observations, Colonel H A GILKES, T A R O, M C, A D M S, Northern Sub-Area, for permission to carry them out, and Brigadier R P CORMACK, O B E, for permission to publish this article.

10 grains, dil. sulphuric acid 10 minims and water to 1 oz.). This was followed by atebirin (Bayer) 0.1 gramme t.i.d. for 5 days, and then plasmoquine (Bayer) 0.01 gramme for 3 days was given to every other case in which crescents were found.

The dosage used for children was quinine sulphate 5 grain t.i.d. for children 6 to 12 years old, 5 grains b.d. for children of 2 to 6 years, and 5 grains daily for infants 6 months to 2 years. The dosage of atebirin used was 6 to 12 years 0.05 gramme t.i.d., 2 to 6 years 0.05 gramme b.d. and 6 months to 2 years 0.05 gramme per day. Plasmoquine was given in doses of 0.005 gramme t.i.d. to children 6 to 12 years, 0.005 gramme b.d. 2 to 6 years old, and 0.005 gramme daily for those 6 months to 2 years. In all cases of the series of observations on all age groups an appropriate dose of sodium citrate was given with each dose of plasmoquine the dose used for adults being 30 grains. In neither series of observations were any toxic symptoms observed nor complained of by patients.

### SERIES I.

In this series of observation on adult Somali males (R.V.B.) a blood film was taken on the 8th day of treatment and if negative was repeated the next day. Every alternate case showing crescents in the peripheral blood on the 8th or 9th day was treated with plasmoquine as above. In both plasmoquine treated cases and control cases blood slides were taken daily until two consecutive slides failed to show gametocytes.

#### SURVIVAL OF CRESCENTS.

In all forty-five cases were so treated. In the plasmoquine group (twenty four cases) the shortest survival time of crescents in the peripheral blood after the 8th day of quinine and atebirin treatment was less than 24 hours and the longest 8 days. The series was 4 3 4 1 4 1 3 4 8 3 4 4 1 3 2, 1 2 4 2, 1 6, 1 days. The mean survival period was 3 days over all cases. (The coefficient of variation = 4 per cent.)

In the control group of twenty-one cases receiving quinine and atebirin only shortest time of survival after the 8th day was 3 days and the longest 16 days. The case series was 3 6, 4 12, 3 9 8 1, 10 13 16, 13 4 15 6, 5 5 15 13 5 7 days, giving a mean survival period for all cases of over 9. (The coefficient of variation = 38 per cent.)

### SERIES II.

In this series of observations every alternate case showing crescents on the day after the completion of the quinine and atebirin course was given plasmoquine in the dosage outlined above, i.e. 1 day of rest was given in all cases before plasmoquine was commenced. If patients did not show crescents on

the 9th day slides were taken morning and evening for 4 days (In only one of the 110 cases did a patient not having crescents in the blood on the 9th day show crescents at a later period In this case very scanty crescents were found on the morning of the 11th day and at that time only)

In all cases, the criterion for disappearance of crescents was eight consecutively negative morning and evening slides This was excessive and unnecessary, for in only one case did crescents reappear after an absence of 36 hours It seems, therefore, that negative slides for 48 hours which was the criterion used in Series I is adequate All slides in this series were examined by one of us (G W A D) and a double check was performed independently by an African laboratory assistant All slides were serially numbered and the numbers changed from time to time so that no association could be made between slide and patient

#### SURVIVAL OF CRESCENTS

The length of survival of crescents in this series is given as from and including the 10th day, *i.e.*, the day following the day of rest on the completion of quinine and atabrin therapy, on which day plasmoquine was given to each alternative case with no selection in regard to age or sex In the plasmoquine treated group there were twenty-two cases with the following time of survival of crescents in the peripheral blood in days 3, 2½, 2½, ½, 1, 3½, 2½, 1, ½, 1, 3, 3, ½, 2, 3, 2, 3, 3, 2½, 2, 3, 3, the average mean survival period being 2 days (The coefficient of variation=47.7 per cent) In only one case did crescents persist after the day on which the plasmoquine course was finished, *i.e.*, the case showing crescents for 3½ days

In the control group there were nineteen cases and the survival time of the crescents was as follows in days 15, 3, 4, 15, 3, 5, 7, 17, 7½, 3, 12, 17, 7, 19 plus, 12, 20 plus, 4, 24, 14, the average mean survival period being over 11 days (The coefficient of variation=60 per cent)

The average survival rate of crescents in each age group is shown in the following table —

	Up to 11 years	12-29 years	30-49 years	50 onwards
Number of cases in plasmoquine group	10	10	1	1
Average survival of crescents in days	2	2	3	½
Number of cases in control group	8	8	2	1
Average survival of crescents in days	10	10	24	12

pathology of leprosy in view of the strong tendency towards natural recovery in mild neural types, which rarely become lepromatous. In the latter form the strong cellular resistance of the neural type is lacking. In recent years, the work of MITSUDA, DHARMENDRA, LOWE, and others on the lepromin test has confirmed the tissue reactions in leprosy as a form of allergy. FRITZ (1943) points out that hyperergic inflammation makes old lesions appear like new ones.

Under these circumstances, the classification of leprosy by PARDO-COSTELLO and TIAH (1943), which has been favourably received in South America and in the United States, deserves consideration. A summary of this is given below —

- 1 *Lepromatous*—with the presence of numerous bacilli and negative lepromin test.
- 2 *Tuberculoid*—with only rare bacilli and positive lepromin test.
- 3 *Non-specific*—with few bacilli and about equal proportions of positive and negative lepromin reactions and with a tendency to develop into either lepromatous or tuberculoid forms.

The non-specific lesions include erythematous, pigmented and achromic forms, with slight enlargement of the peripheral nerves. The tissues show simple inflammatory changes characterized by lymphocytic accumulations round the blood vessels and small nerves. These authors recommended for the differential diagnosis of this type of case that the skin should be pricked with a needle through a drop of 1 in 1,000 histamine. The occurrence of an erythematous halo around the rapidly forming wheal indicates that the nerve endings are intact and that the case is not one of early leprosy. In positive cases, the prognosis may be foretold by means of the lepromin reaction.

#### LEPROSY AS A MILITARY PROBLEM IN INDIA.

According to the *British Empire Leprosy Relief Association (India Council) Annual Report 1941* it is estimated that there are over one million lepers in India, if early cases are included. In some areas the endemicity is as high as 5 to 10 per cent. of the population. Infective cases number about 250,000 of which about 14,000 are cared for in institutions (this number includes many non-infective crippled nerve cases). In Bengal 4 per cent. of the police force show signs of leprosy practically all in a mild form.

Before the war when recruiting was largely limited to Northern India, where endemicity of leprosy is low except in certain hill regions it would appear that there were few military lepers. The expansion of the army and recruiting on an all-India basis have materially altered this position. In military practice, the differentiation of infective and non-infective lepers is not practicable and discharge from the army must be effected as soon as the

diagnosis is made. The differential diagnosis between early cutaneous leprosy and other skin lesions, such as leucoderma, is notoriously difficult, and unless the medical officer has a clear understanding of both clinical and laboratory diagnostic measures in leprosy, patients will continue to occupy badly needed hospital beds for an undue period.

## METHODS OF LABORATORY DIAGNOSIS

### A BACTERIOLOGICAL

Smears should be taken from the skin by the standard technique recommended by ROGERS and MUIR (1940) and stained by the Ziehl-Neelsen method. This is the method of choice in the lepromatous form of the disease and in the major tuberculoid form of nerve leprosy. In the minor tuberculoid forms and in simple leprides, recovery of the bacilli is a rarity, and this procedure is a waste of time. In doubtful neural cases with thickened nerves, the epineurium should be incised longitudinally and a smear made from a few teased fibrils.

### B LEPROMIN TEST

The antigen for this test is defined by FERNANDEZ and OLMOS CASTRO (1942) as —

- (i) *Whole lepromin* This contains all the constituents of a leproma-bacteria, cells and tissue detritus, and is the type originally used by MITSUDA.
- (ii) *Bacillary lepromin* An antigen composed of a suspension of bacilli.
- (iii) *Lepromin filtrate* The soluble active substances of *M. leprae* obtained by filtration or chemical extraction and containing no acid-fast bacilli.

The response to whole lepromin is (a) an early erythematous infiltration reaching its acme in 48 hours, beginning to decline in 72 hours and disappearing in a week, (b) a papular or nodular lesion, commencing about the 7th day and reaching its maximum in 3 to 4 weeks after injection.

FERNANDEZ and OLMOS CASTRO compared reactions in lepromatous and tuberculoid cases and in supposedly healthy persons using whole lepromin, lepromin filtrate prepared by their own technique and lepromin filtrate prepared according to the DHARMENDRA (1942) method.

*Whole lepromin* Both early and late reactions were negative in the lepromatous cases, were frequently positive in the neural forms and were more so in the tuberculoid forms. In healthy persons the early reaction was almost invariably negative, while the late reaction was positive in 38 per cent. Positive early reactions could be obtained by previous sensitization with whole lepromin. It is stated that there is a positive early reaction in tuberculous patients not suffering from leprosy.

*Lepromin filtrate* Early reactions similar to those obtained by whole lepromin were obtained. The late reaction did not occur.



## C. SEROLOGY

HOPKINS and FACET (1944) draw attention to the fact, not generally appreciated, that the Wassermann and Kahn reactions are frequently positive in leprosy syphilis being absent. In 693 cases of all forms of leprosy 287 (41.4 per cent.) gave a positive reaction. They state that there is an increase or decrease of reaction in one or both tests which is closely related to the clinical manifestations of the disease.

## D. SKIN BIOPSY

According to ROGERS and MITCHELL (1940) this is only justified in cases in which bacteriological examinations are negative and the clinical signs are doubtful. In such cases there is as a rule to be seen in sections of the skin little that is clearly pathognomonic of leprosy as compared with other skin diseases. It should be realized, however that the vast majority of military lepers fall into this category. As will be shown below the information derived from skin biopsy gives a higher percentage of confirmed diagnoses than can otherwise be obtained, since lepromin is not usually available in military practice.

The interpretation of skin biopsies from suspected lepers is a difficult matter except in lepromatous cases and can only be undertaken by an expert histologist. It is essential for the latter to maintain a close liaison with the clinician. In the presence of a clinical leproide, the histological appearances of the major tuberculoid type are sufficiently characteristic to afford absolute confirmatory diagnosis in the majority of cases. In the minor tuberculoid type the findings are rather less obvious but are nevertheless of diagnostic significance.

Tuberculosis cutis cannot be differentiated with absolute certainty on purely histological grounds here the clinical appearances must serve as a guide to the interpretation. In doubtful cases, a guinea-pig must be inoculated with an emulsion of the skin lesion.

Whenever nerve fibrils can be identified in the section, the chances of making a positive diagnosis of leprosy are greatly enhanced. CASTANIÉ DECODÉ (1942) stresses the importance of neuritis in the histological differentiation of leprosy from other tuberculoid granulomata. He describes a characteristic "para-arterial neural infiltration" which commences round the nerve fibrils but does not invade the vascular structures. As the disease advances destruction of nerve fibrils renders impossible this diagnostic aid. COCHRANE (1943) points out that when large nerve trunks become involved by the tuberculoid reaction, "abscesses" may form within the nerve sheaths causing irreparable destructive lesions. Apart from a minor degree of endarteritis obliterans vascular changes are not obvious except in the rare Lucio leprosy where MARTINEZ BUZ (1942) describes an acute endovascularitis. The main diagnostic features of fully



FIG 1 Skin of arm, fully developed tuberculoid leprosy. An epithelioid and giant cell reaction is occurring in the subcutaneous tissue. Nerve fibres are undergoing infiltration and atrophy. H & E 60

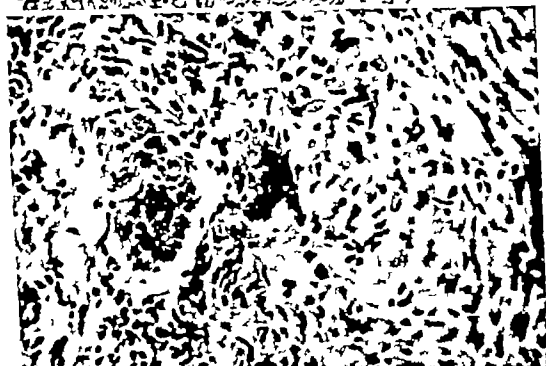


FIG 2 Lower left hand of field in Fig 1,  $\times 275$ . A degenerate nerve fibre is seen surrounded by epithelioid and round cell system, with a giant cell of "foreign body" type close to it.



FIG 3 Skin of arm, early tuberculoid leprosy. Nerve fibres are easily identified but there is commencing round cell perineuritis. H & E  $\times 80$



developed tuberculoid leprosy (Figs 1 and 2) as compared with tuberculosis cutis are summarized as under —

Epidermis

Dermis and subcutaneous tissue

#### TUBERCULOID LEPROSY

A degree of epithelial atrophy and depigmentation are usual

Oedema not marked Relatively vascular Epithelioid cell reaction both superficial and deep in early cases Giant cells of "foreign body" type True caseation very uncommon Typical destructive perineuritis and endoneuritis

#### TUBERCULOSIS CUTIS

Acanthosis and hyperkeratosis are common

Oedema marked Fully developed lesions relatively vascular Epithelioid cell reaction usually limited to deeper layers in early cases Giant cells of "Langhans" type A degree of caseation not uncommon Nerve fibrils not specially affected

An early case of minor tuberculoid leprosy is shown in Fig 3 Epithelioid cell reaction is minimal but perineuritis is distinct The non-specific leprides show no characteristic histological structure and reliance must be placed on the histamine and lepromin tests

Skin biopsy as a routine diagnostic measure was introduced in 1942 by Major L. KRAINER, R A M C, at the Southern Army Laboratory, India

#### ANALYSIS OF SKIN BIOPSIES FROM SUSPECTED LEPERS AT THE CENTRAL MILITARY PATHOLOGICAL LABORATORY, INDIA

During the period 20th August, 1943, to 31st September, 1944, 174 biopsies were received from hospitals in the Poona Kirkee area

#### 1 TECHNIQUE

(a) *Selection of specimens* A wedge of skin from the suspected lesion, at least  $\frac{1}{2}$  by  $\frac{1}{8}$  inch in area and  $\frac{3}{8}$  inch in depth is removed under local anaesthesia Length is fully as important as depth since the nerve fibrils are irregularly distributed

(b) *Preparation of specimen* This is fixed in 10 per cent formal saline for 12 to 24 hours and left in 95 per cent alcohol overnight Then transfer to absolute alcohol for 4 hours followed by chloroform for 4 hours and leave in a saturated solution of paraffin wax in chloroform at 37° C overnight Embed in paraffin wax (one change) for 8 hours Sections are prepared in the usual manner Staining by routine Ehrlich's haematoxylin and eosin is perfectly satisfactory for nerve fibril differentiation owing to the eosinophilic properties of the latter but dehydration of the section in high grade alcohols must be carefully controlled under the microscope having first identified a large nerve fibril Beautiful results are obtained by staining with Weigert's iron haematoxylin followed by Ponceau 2 R acid fuchsin and counterstaining with light green or very dilute Masson's aniline blue The deep pink nerve fibrils stand out against a green or blue background

## 2. TABULATION OF FINDINGS.

	Number	Per cent
Positive leprosy	80	46.0
Doubtful leprosy	14	8.0
Other lesions	6	3.4
No diagnosis	74	42.5

Among the positive leprosy cases, seventy-nine were of tuberculoid type (Indian) and one was lepromatous (a West African). Acid-fast bacilli were demonstrated in a few of the tuberculoid specimens, but Ziehl-Neelsen staining was not adopted as a routine, since tuberculous cutis cannot be differentiated from leprosy in such cases by this means.

The six other lesions comprised molluscum contagiosum, psoriasis, psoriasisform syphilide, lichen planus, pityriasis, rubra pilaris and mycosis fungoides.

During the period covered by these biopsies 276 suspected lepers were admitted to military hospitals in the Poona-Kirkee area and 234 were medically boarded and discharged from the Army as lepers. (Fifty-seven of these were West Africans and the remaining were Indians.) It is not possible to state how many diagnoses were confirmed by demonstration of acid-fast bacilli in smears or by examination of tissue sections. The eighty positive cases described above however appear to represent a fair proportion of these diagnosed as lepers during this period and it is unlikely that confirmation of the diagnosis by examination of smears could have been effected.

## SUMMARY

- 1 The clinical and pathological classification of leprosy is briefly described.
- 2 The methods of laboratory diagnosis are discussed with particular reference to skin biopsy.
- 3 An analysis of 174 biopsies from suspected lepers is given, the diagnosis having been confirmed in eighty cases.
- 4 It is suggested that skin biopsy affords the most rapid confirmatory evidence of the tuberculoid type of leprosy most frequently encountered in military practice in India.

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## THE METABOLISM OF RAT LEPTOMAS AND OF RAT LEPROSY BACILLI \*

BY

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Lepromas of rats heavily infected with rat leprosy were removed by biopsy parts were used for histology and parts for the study of oxygen consumption, aerobic and anaerobic glycolysis in a Bancroft-Warburg apparatus according to Warburg's methods. Histologically these lepromas showed a uniform picture, they consisted of masses of cells stuffed with bacilli and with nuclei containing relatively little chromatin and indistinguishable from reticulum cells. There were practically no other types of cells, *i.e.*, no round cells or large mononuclears. Irregular patches of necrosis with broken up nuclei were present throughout the lepromas and these necrotic patches consisted mainly of masses of bacilli. After determining the gas exchange the tissues were removed and their nitrogen content determined in a micro-Kjeldhal apparatus. The final results (Table I) were expressed in c mm per hour per milligram N. As can be seen from the table the lepromas consume oxygen, but there is practically no glycolysis aerobic or anerobic. The respiratory quotient varied from 0.56 to 1.0.

Heavily infected lepromas from rats and from the liver of a heavily infected hamster were ground up in sand, saline was added, the mass stirred and slowly centrifuged for 3 minutes. The deposit was discarded and supernatant fluid was removed and again slowly centrifuged. This was repeated three times, and finally after rapid centrifugation the deposit consisted only

\*This work was carried out with a grant from the National Medical Research Council

of bacilli. These bacilli freed from tissues were placed in a Bancroft Warburg apparatus, the respiration determined and expressed in c.mm. per hour per milligram nitrogen as determined in a micro-Kjeldhal. As can be seen from the table the bacteria freed from tissues consume oxygen, but there was no evidence of glycolysis under aerobic and anaerobic conditions.

TABLE I  
METABOLISM OF RAT LEPTOMAS

Oxygen consumption in Ringer and bicarbonate in mm. <sup>3</sup> O <sub>2</sub> per hour per mg. N	Aerobic glycolysis in Ringer bicarbonate and glucose in mm. CO <sub>2</sub> per hour per mg. N	Anaerobic glycolysis in Ringer bicarbonate and glucose in mm. CO <sub>2</sub> per hour per mg. N.	R.Q.	
8	+ 1.2	0	—	Leptoma rat 145a
14	0	0	—	Leptoma rat 84 11
13	0.3	+ 1.6	0.87	Leptoma rat 146a
13.7	0	0	1.1	Leptoma rat 84 12
3.4	0.5	1	0.56	Leptoma rat 146a
3.3	0	0	—	Leptoma rat 146a
7.8	0	0	0.92	Leptoma rat 162a

METABOLISM OF ISOLATED RAT LEPTOSY BACILLI

3.1	+ 0.6	+ 0.8	1.1	Leptosy from liver of hamster 84 13
12.6	0	0	0.62	Leptosy from leptoma rat 162a
8.4	0	0	1	Leptosy from leptoma rat 190a

# SUMMARY

Heavily infected rat leptomas containing cells of a uniform type stuffed with rat lepra bacilli show oxygen consumption but no aerobic or anaerobic glycolysis.

Bacilli liberated from tissues also show oxygen consumption but no aerobic or anaerobic glycolysis.

# STUDIES IN LEISHMANIASIS IN THE ANGLO-EGYPTIAN SUDAN

## VIII SOME OBSERVATIONS ON THE CHEMOTHERAPY OF KALA-AZAR \*

BY

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### INTRODUCTION

SIR LEONARD ROGERS (1939) has pointed out that the use of antimony in the treatment of leishmaniasis is one of the most successful achievements of modern chemotherapy, and merits rather wider attention than it has received. The literature referring to the chemotherapy of leishmaniasis is certainly small compared, for example, with that referring to syphilis. This is probably because the latter infection includes in its geographical distribution the temperate climates in which there has been the most intensive development of scientific medicine, whereas the former is largely restricted to tropical and

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subtropical areas. Nevertheless the area of the world's surface in which leishmaniasis occurs is large. The infection may be as protean in its manifestations and as interesting in its wealth of clinical material as syphilis, while in some forms it is more deadly in its results. The present writer believes also that kala-azar is a disease of unique interest from the point of chemotherapy for several reasons.

1 In the first place it is one of the diseases for which effective chemotherapeutic agents are now on the market in considerable variety.

2 Secondly kala-azar is known to be a very fatal disease in the absence of treatment. In diseases like pneumonia or gonorrhoea it may be difficult to assess the value of a new specific chemotherapy because previous results obtained by other methods of treatment were often good. This is not so in kala-azar because the older methods of treatment had no success in this disease. Its course was completely unaffected by any of the numerous remedies which were tried before the introduction of antimony (CARSON, 1930). Therefore a small number of cases is generally sufficient to demonstrate whether any new form of chemotherapy has any action or not. It is rather a different matter of course, if the assessment of the relative values of several effective remedies is required.

3 Although kala-azar is a fairly chronic disease, with many clinical variations, there need not now be any doubt about the final result of treatment in any case. The patient either recovers or dies, it is not possible to carry on for years suppressing the more urgent manifestations by treatment without knowing whether a true cure has been achieved, as may be done for example in syphilis. The ultimate test of cure in kala-azar is a simple one—the absence of relapse within a limited period after the completion of treatment. Experience shows that the period of follow-up required to ascertain whether relapse will occur or not is comparatively short—NARVER (1932) places it as low as 6 months in Indian kala-azar.

4 Finally it has been possible to recognize in a proportion of cases that under the influence of chemotherapy the infection may pass through a fairly specific course of evolution which is of great theoretical interest.

Thirty years have elapsed since kala-azar became a curable disease. During this period improvements in technique combined with the introduction of less toxic and more effective drugs have resulted in a further reduction in the mortality rate until in the latest series reported from India by NARVER and his colleagues (1942) it was less than 2 per cent. The fact that the disease has proved less amenable to treatment in other places detracts nothing from this remarkable achievement. With the development of specific chemotherapy the prognosis has been profoundly altered in kala-azar everywhere. In the Sudan, where the disease appears to be more resistant to treatment than in India, a recovery rate of over 70 per cent. can now generally be obtained with-

out great difficulty. The problem which now remains is that of eliminating the residual mortality rate of 25 to 30 per cent by the further perfection of chemotherapeutic methods.

The present communication is a record of work directed to this end. It is essentially a summary, which reviews briefly the writer's experience during the past 10 years and refers also to that of previous workers. If the results have been less successful than was anticipated, the record is still of some interest in view of the fact that investigations undertaken primarily to improve the disappointing results of treatment in Sudan kala-azar have thrown some light on (1) the manner in which drugs act to bring about cure in this disease and (2) the natural history of leishmania infections in the human host (cf KIRK, 1944). Perhaps it should be pointed out that this article is much coloured by the writer's own views on these points, with which other workers may not agree.

This paper makes no attempt to give detailed practical instructions for the guidance of others who may have to treat cases of kala-azar. It is submitted in the belief that the record is not without interest merely as an essay on the practice of chemotherapy in a specific disease in man.

#### ANTIMONY

According to CHOPRA (1936) the use of antimony in kala-azar was first suggested by SIR PATRICK MANSON, but GASPAR VIANNA (1912) was the first to try intravenous injections of tartar emetic, with which he successfully treated South American forms of cutaneous and muco-cutaneous leishmaniasis. Confirmation of his results was not long delayed. In Italy, DI CRISTINA and CARONIA (1915) found the drug effective in infantile kala-azar, while in the same year ROGERS (1915) used it in India, to be followed quickly by MUIR (1915) and MACKIE (1915).

During the next 5 years the sodium salts were substituted for the potassium salts (ROGERS, 1918), and a number of purified brands suitable for intravenous injection were put on the market. Otherwise little change was made in the treatment of the disease in India. Little change was required according to ROGERS (1939) the use of antimony by McCOMBIE YOUNG in the Sibsagar epidemic of 1921 resulted in a recovery rate of 88 per cent, as compared with 4 per cent in the earlier Nowgong epidemic. There is probably no better achievement in the history of chemotherapy.

The first pentavalent aromatic compound of antimony—sodium paracetyl-amino-phenyl stibiate, or "stibacetin"—was introduced by the firm of VON HEYDEN in Germany. Intramuscular injections of this drug were used with success by CARONIA (1916) in infantile kala-azar. The work was followed up by BRAHMACHARI (1922) who prepared a number of pentavalent compounds including urea stibamine which was later found to be effective therapeutically.

Compared with tartar emetic the pentavalent compounds had some notable advantages. Immediate reactions such as coughing and joint pains were eliminated and pneumonia, one of the most frequent causes of death during treatment, was greatly lessened. The total amount of antimony necessary for cure could be administered in a much smaller number of doses, so the length of treatment was reduced. It was found that resistant cases which responded slowly when treated with the tartrates improved rapidly with larger doses of antimony in the form of the less toxic pentavalent compounds. Finally the drug could be given intramuscularly a point of practical value in the treatment of young children in whom suitable veins are often difficult to find. A large number of pentavalent antimony preparations is now available and they have been universally used in kala-azar with excellent therapeutic results. It is probable that the series is still by no means exhausted.

From the beginning the results obtained with antimony in Sudan kala-azar were disappointing as compared with those reported from India. Previous attempts had been made to treat the Sudan disease with senega (EKSON, 1909), "606" (BALFOUR and ARCHIBALD 1911), vaccines (ARCHIBALD 1913), and other methods but MARSHALL (1912) stated that "no drug has been found which in any way affects the course of the disease, and all cases seen since our last report have terminated fatally." It is a little surprising that antimony was not tried at an earlier date in the Sudan, since at the instigation of PLINDER the action of this drug in trypanosomiasis was extensively investigated. Following the demonstration by PLINDER, FRY and RANKEN (1910) that animals infected with trypanosomiasis could be cured by injections of metallic antimony RANKEN (1913) records that in May 1910 Major FRY gave an intravenous injection to an advanced case of kala-azar and thus demonstrated that the treatment was applicable to man. Thereafter extensive clinical trials of antimony in human trypanosomiasis were carried out in the Southern Sudan by RANKEN, FRY, THOMPSON and others, who were favourably impressed with the results. Had it not been for the war in which Major RANKEN was awarded a posthumous V.C. in 1914 it is possible that antimony might have been tried in Sudan kala-azar at an earlier date. As it was, the earliest attempts to treat the Sudan disease were carried out along the lines which had previously been found so effective by Indian workers in Indian kala-azar. The results were exceedingly variable and much less satisfactory than those which had been obtained in India. Thus ARCHIBALD (1923) cites a series of fifteen cases with thirteen deaths in which he attributes many of the fatalities to toxic effects of the drug rather than to the disease. However there was no doubt that some cases even in an advanced stage of the disease benefited from the treatment and were cured. Two different points of view can be recognized in the early publication from the Sudan on this subject. CHRISTOPHERSON (1921), describing cases studied and treated personally by himself, regarded antimony as a safe and reliable specific in leishmaniasis, provided caution and judgment were used in its administration, but

pointed out that large total doses were sometimes necessary for cure. On the other hand, ARCHIBALD (1923) while admitting that antimony was the only treatment of any value in kala azar laid stress on the toxic effects and the disappointing results obtained as a routine in Sudan hospitals and out stations as compared with those reported from India.

During the following 20 years the comments on this subject in the *Annual Reports of the Sudan Medical Service* (1925-1942) are exceedingly variable, and the reported immediate mortality rate changes from 40 to 20 per cent.

#### IMPORTANCE OF GENERAL TREATMENT AND SUPERVISION

FLETCHER and JEPPI (1924) in their studies in dysentery in the Federated Malay States record an interesting experience which at one time seemed to the writer to have an important bearing on the variable results obtained in the treatment of Sudan kala azar. During a preliminary investigation in the autumn of 1920 these authors were greatly surprised to find that the routine hospital treatment with emetine was of no benefit in Tamil labourers with amoebic dysentery. Indeed it appeared to do harm rather than good. The patients who in addition to having amoebic dysentery were usually in an extremely advanced stage of malnutrition and exhaustion, became progressively worse and died in spite of its administration while blood, mucous, and tissue invading forms of the amoeba were found in repeated examinations of their stools. Those results were so contrary to FLETCHER and JEPPI's own experience of the drug elsewhere that they could not help wondering if there were some mistake in its administration or compounding. But apparently no mistake was found, since none was recorded. The drug was obtained from a first class firm, compounded in the hospital dispensary, and administered by one of the hospital dressers. However FLETCHER and JEPPI decided to have some cases treated under their own supervision, and in the following year they treated a series of seventy-eight cases controlling their treatment by periodic examination of the stools. In seventy-seven of the cases the acute symptoms subsided under emetine treatment, while blood, mucous and amoebae had disappeared from the stools by the time twelve injections had been given thus showing that in the hands of FLETCHER and JEPPI the specific action of emetine was as manifest in Tamil coolies as in any other group of people.

Most people who have worked for some time in tropical hospitals can probably recall similar experiences, for which it was difficult to state the precise explanation. The reduction of treatment to a standard course of injections, the empirical administration of which can be delegated to subordinates, always entails the risk that results will deteriorate, especially with toxic drugs like emetine or tartar emetic, to which individual tolerance varies greatly. CHRISTOPHERSON (1919) stressed this point in the use of tartar emetic in schistosomiasis, while ARCHIBALD (1923) maintained that many of the early fatalities in the

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is not apparent in the earlier publications, had been the frequent occurrence of relapse in patients discharged from hospital as cured. There is no doubt that this was simply the result of insufficient treatment. Some case-histories published by SOMERS (1944) illustrated this point very clearly. In the early days no satisfactory criteria of cure were available, and it was often accepted that if a patient had received the treatment advocated by experienced workers in India he could be presumed cured or, alternatively, be regarded as "antimony resistant" if treatment had produced no improvement. We, therefore devoted attention to the question of relapse by following up patients after discharge from hospital and attempting to correlate the occurrence of relapse with the amount of treatment which had been given and the tests used as evidence of presumptive cure. As a result of this enquiry it was possible to work out some fairly reliable criteria of presumptive cure, and these are discussed later. At the same time useful information was gained about the amount of treatment required for cure in Sudan kala azar.

#### ANTIMONY TREATMENT IN SUDAN KALA-AZAR

In the primitive and rather inaccessible endemic areas it is difficult to provide facilities for the nursing and close clinical observation required in a serious disease like kala azar, but by such attention to those matters as was possible a definite improvement in the results of treatment was obtained, until the immediate cure rate reached a comparatively steady figure of approximately 77 per cent. Illustrative results have already been reported by my colleague, Dr MOHAMMED SATI (1942). The War Office publication, *Memoranda on Medical Diseases in Tropical and Subtropical Areas* (1942) states that Sudan kala azar does not react to antimony, but SATI's figures show that this is not entirely correct.

It may be noted that our experience in this matter has been very similar to that previously recorded by HENDERSON (1937), who treated over 300 cases of Sudan kala azar during a period of 3 years (1933 to 1936). HENDERSON records that in the first year of his work the death rate was as high as 50 per cent, but with increasing experience and clinical study it was steadily reduced, until in the latter part of the series it was 25 per cent. COLE (1944) in East Africa has recently recorded a similar decline in mortality rate with increasing experience of the disease.

It must be admitted that even the best results which have been achieved in the Sudan still compare unfavourably with those reported from India, where an appreciably higher rate of cure is the rule. Probably it will be difficult to obtain any further reduction in the mortality rate in the Sudan under present conditions owing to the fact that in every series there must be included a number of patients who apply for treatment only in the very last stages of the disease and are practically moribund on admission to hospital.

treatment of Sudan kala-azar were due to overdosage of the drug as a result of employing the Indian standard doses empirically in individual obviously intolerant to the toxic action of tartar emetic.

The present writer became interested in kala-azar from the point of view of aetiology rather than as a clinician, but it seemed that the best start was to be made by following up ARCHERDALL'S attempts to improve treatment in the endemic areas, because it was the best method of access to material for further study. Also, it seemed possible that there was here a situation not unlike that recorded by FLETCHER and JEPPE. A large proportion of the patients who came for treatment in the endemic areas were in a very advanced stage of the disease having come to the Government hospital and dispensaries only as a last resort, after having previously tried all manner of native medicines and magical cures. Many of them had in addition become reduced to a very poor nutritional state or the disease had become complicated by sepsis and other intercurrent infections, such as malaria, amoebic dysentery or helminthiasis. We had observed that the course of experimental kala-azar in monkeys appeared to be greatly influenced by diet and general living condition (cf KIRK, 1944). ADLER, TITUSON and WITTENBERG (1938) had previously noted the same thing in canine kala-azar. It seemed therefore, that some improvement might be achieved by devoting attention to general treatment such as improving the diet, and ascertaining the best methods and most favourable times for dealing with intercurrent infections. It was noted also that in many of the fatal cases death was often the immediate result of one of the complications or acute emergencies which arise from time to time in the course of the disease, such as pneumonia or diarrhoea. Specific anti-kala-azar treatment alone may be insufficient to deal with such emergencies, but if they are recognized early and vigorously treated on general or symptomatic lines it is often possible to tide the patient over the emergency.

In addition to specific treatment we devoted special attention to general and symptomatic treatment. Patients were given a nutritious, high-protein diet, which included also fresh vegetables and a daily ration of raw liver. Attention was paid to oral hygiene and regular mouth washes were instituted. An iron and arsenic tonic was prescribed as a routine for the anaemia. Blood transfusion was sometimes used. Without interrupting specific treatment intercurrent infections and serious complications were treated as the occasion arose. Purgation, either as a routine measure at the beginning of treatment or at any other time was sedulously avoided (injudicious purgation is probably the most certain method of precipitating a fatal termination in kala-azar). Diarrhoea was treated symptomatically at the earliest possible moment usually it responded to a stock bismuth mixture if this were given promptly and in sufficient dosage, but if not recourse was had to opiates without delay.

Another disappointing feature in the treatment of Sudan kala-azar which

stressed the importance of early diagnosis and early treatment in kala-azar at any rate in the Sudan variety. We have seen many cases in which intercurrent conditions, like malaria, amoebiasis and helminthic infection, have delayed diagnosis for months until the patient was in an almost moribund condition, then finally specific treatment was instituted, and he died after the first few injections.

It is often stated in the literature that late cases of kala-azar react better to treatment than do early cases. There is theoretical evidence to suggest that this should be so in some cases, but in practice we have never been able to confirm it. All our experience points to the conclusion that the converse is more generally true in Sudan kala-azar.

#### RELATIVE RESISTANCE TO TREATMENT OF DIFFERENT TYPES OF KALA-AZAR

In spite of the efforts we have described the results of treatment in Sudan kala-azar still remained greatly inferior to those which had been obtained in India. Indeed, all that had been achieved was a slight reduction in the mortality rate to a fairly constant figure, and the assurance that the cases had been adequately treated and, as far as we could ascertain, did not relapse soon after discharge from hospital. Apparently the problem required rather a different solution from that of FLETCHER and JEPPE.

Facilities for clinical study and treatment are much better in India than in the Sudan, and it is possible that this may partly explain the difference in results. It is unlikely, however, to be the whole explanation. Nor can the disappointing results of treatment in the Sudan be ascribed wholly to primitive conditions in the endemic areas, or the effects of malnutrition. Intercurrent infection with malaria, helminthiasis and dysentery, or to the fact that patients often come for treatment only after the illness has reached an advanced stage. Dr. SEV GUPTA (1944) informs me in a letter that almost similar conditions prevail in the rural areas in Bengal, yet there the disease responds readily to antimonials and 90 per cent of cases are cured by one course of injections.

The observations of BRYANT and FAIRMAN (1939) indicate that different peoples may react very differently to various drugs and diseases, and the possibility cannot be excluded that the relative resistance to treatment of Sudan kala-azar is due to some "host factor" inherent in the Sudanese peoples. We have unfortunately no data to offer on the question of racial resistance or susceptibility in kala-azar.

We have previously put forward the suggestion (KIRK 1942) that the parasites of Sudan kala-azar are different from those of the Indian disease because a different vector is concerned in transmission and the latent period of 1 to 2 years seen in India between cure of the visceral disease and the appearance of post-kala-azar dermal leishmaniasis is not seen in the Sudan where the dermal manifestations usually appear as the visceral disease subsides.



Another difficulty for which we have no explanation at present is the occasional occurrence of sudden death, for no apparent reason, in a patient who appears clinically to be doing well. The observations of MAINZER and KRAUSE (1940) suggest that such sudden deaths may be due to a toxic action of antimony on the myocardium, but at present we do not really know their cause.

In the prolonged period of hospitalization and the large total doses of antimony required for cure the results are also much inferior to those obtained in India, where a large proportion of cases can apparently be cured by an intensive course of eight to ten daily injections given in the out-patient department. From the experiences described in the preceding section of this paper it will be evident that in the Sudan kala-azar is not at present a disease suitable for out-patient treatment. With the methods used at present an average period of hospital treatment of about 3 months is required for cure, and approximately three times the total dosage of pentavalent antimony which I found sufficient for cure in Indian kala-azar. Individual cases are encountered in which cure is achieved much more easily than this but these are compensated by others which require even more prolonged treatment.

It may be added that a small proportion of Sudan cases seem to be completely resistant to any form of antimony treatment. Clinically such cases are usually of a fairly chronic type with an indefinite history as regards duration. Parasites may be scanty or difficult to find in spleen punctures, but sometimes they are found easily and in large numbers. We have been able to follow one or two cases of the latter type to postmortem examination and verify that, even after prolonged and intensive treatment with antimony *leishmania* were still present in large numbers in all the principal tissues, thus indicating that in these cases, the drug had failed to affect the course of the infection.

As regards the drugs used in treatment, it was our opinion before the introduction of stilbamidine that of the antimonials neostibosan was the preparation of choice in kala-azar. Solustibosan we found less toxic but less effective in the recommended doses which had a relatively high antimony content. Indian workers (NARAYAN and SEN GUPTA 1942) appear to have reached a similar conclusion, but other observers do not all concur on this point. In the Sudan, Dr R. W. STEPHENSON (1940), working under different conditions from those with which we are familiar found urea stibamine a more efficacious drug than neostibosan, especially in cases with cancerum on the spleen. After an extensive review of the subject, CAROTTA (1930), who was the first to use any of the pentavalent compounds of antimony says he is not convinced that these are any better than tartar emetic used with care and experience.

From these divergent opinions it may be suggested that choice of a particular drug or preparation is not the main factor influencing the results of treatment. We believe that the experience and clinical judgment of the physician count for much. This includes diagnosis. SMITH (1942) has rightly

stressed the importance of early diagnosis and early treatment in kala-azar at any rate in the Sudan variety. We have seen many cases in which intercurrent conditions, like malaria, amoebiasis and helminthic infection, have delayed diagnosis for months until the patient was in an almost moribund condition, then finally specific treatment was instituted, and he died after the first few injections.

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We have previously put forward the suggestion (KIRK, 1942) that the parasites of Sudan kala azar are different from those of the Indian disease because a different vector is concerned in transmission and the latent period of 1 to 2 years seen in India between cure of the visceral disease and the appearance of post-kala azar dermal leishmaniasis is not seen in the Sudan where the dermal manifestations usually appear as the visceral disease subsides.

It may be suggested that the relatively greater resistance of the Sudan disease to treatment is the expression of a further point of difference between the two varieties of kala-azar. A review of the literature gives some support to this view. SCHMIDT and PETER (1938) have compiled a very full summary of the literature from different countries relating to treatment with antimony. From such a review it appears that Indian kala-azar is more amenable to treatment with antimony than other varieties and that experiences in the other endemic centres have been similar to those in the Sudan rather than to those in India. Thus in Chinese kala-azar it is stated that the action of antimony is slower than in Indian kala-azar while the clinical picture of the disease is graver, agranulocytosis and complications affecting the mouth, throat and lungs being especially frequent and severe. Chinese patients are said to be particularly sensitive to antimony so that toxic reactions are common, the death-rate is high, and prolonged hospitalization is necessary for cure, even with the pentavalent compounds.\* Mediterranean kala-azar also appears to be much more difficult to influence with antimony than the Indian variety. Here also the Indian treatment has proved inadequate and larger total doses are generally required. References to "antimony resistant" types of the disease are frequent in papers from the Mediterranean region. ADLER and TCHERNOMORETZ (1941) state that laboratory observations on Syrian hamsters show that aromatic antimony compounds are far less effective in Mediterranean than in Indian kala-azar, a finding in conformity with the general experience in Mediterranean countries that about three times as much urea stilbamine or neostilbosan is required to cure a Mediterranean case as was found sufficient by Indian workers for Indian kala-azar.

It is very interesting to observe in passing that from a review of the literature one gains the impression that South American muco-cutaneous leishmaniasis is the most resistant to treatment of all forms of leishmania infection. Nevertheless it was in this form of leishmaniasis that GASPARD VIANNA first recognized the specific action of antimony from which began the chemotherapy of kala-azar.

#### THE DIAMIDINES.

The first attempt to treat human leishmaniasis with the diamidines was made by ADAMS and YORKE (1939), who administered stilbamidine (4,4-diamidino stilbene) in a case of Indian kala-azar in Liverpool, and obtained an apparent cure after eight daily injections of 1 mg. per kg. body weight. Shortly afterwards ADLER and RACHOWITZ (1939) successfully treated a case of Mediterranean kala-azar with the same drug, but in this case a greater

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amount of treatment was required for cure—twenty-two injections of 17 mg per kg, given over a period of 2 months

In August, 1939, Prof WARRINGTON YORKE kindly sent to us samples of stilbamidine, propamidine and pentamidine, with the suggestion that we should try these drugs in Sudan kala-azar. We were very glad to do so, especially as we had now evolved a satisfactory scheme for the general management and supervision of kala azar cases under treatment, which was producing fairly constant results with the drugs available.

During the latter half of 1939 and the first half of 1940 we were able to treat some forty four cases of Sudan kala-azar with the new drugs. Including cases admitted to hospital in a moribund condition, who died after only a few injections there were eight deaths in this series. The remaining thirty-six cases were discharged as provisional cures but like ADLER and RACHMILEWITZ (1939) we found that the amount of treatment required for cure was considerably greater than that found necessary by ADAMS and YORKE in their case of Indian kala azar.

With the extension of the war to East Africa it seemed unlikely that we should be able to continue this work any further. It was therefore decided to publish such results as had already been obtained, since they compared favourably with the results previously obtained by other workers in the Sudan. They were better than the best of our own antimony results, although the difference was not statistically significant. Also, it seemed desirable to record our experience in connection with dosage, methods of administration, toxic reactions, etc. Reference was made in this publication (KIRK and SATI, 1940) to three cases which were apparently resistant to antimony, but improved rapidly when the treatment was changed to stilbamidine. At the time it was difficult to assess the significance of this. The cases were "antimony resistant" according to the usually accepted clinical standards, namely, that they showed no improvement after an amount of treatment which is ordinarily sufficient to effect cure in Sudan kala azar. But while cases undoubtedly occur which are completely resistant to any form of antimony treatment, it is difficult to state that any particular case falls into this category unless the case ends fatally. We have seen cases in the past which were regarded as "antimony resistant" because two or three courses of treatment had produced no reaction, but which improved rapidly after further courses of treatment with the same preparation.

In the same year NAPIER and SEN (1940) published a preliminary report on eight cases of Indian kala azar who were all apparently cured by eight to ten daily injections of 1 to 2 mg of stilbamidine per kg body weight. Further apparent cures were reported by ADAMS and YORKE (1940), ADAMS (1941), WINGFIELD (1941). In the following year NAPIER, GUPTA and SEN (1942) published a detailed account of 101 cases of Indian kala azar treated with stilbamidine with an immediate cure rate of 98 per cent. They concluded that in the ordinary

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case of Indian kala-azar the results compared favourably with those produced by neostibosan, the best drug hitherto used while in antimony resistant cases the results of treatment with stilbamidine were almost as favourable as those in ordinary cases. A number of other workers have subsequently reported cases treated successfully with this drug, while ADAMS (1941) and NAMER and SEN GUPTA (1943) found that pentamidine (4-4-diamidino diphenoxy propane) is also therapeutically effective in kala-azar. ADAMS (1943) has recently reported the successful treatment of a case with intramuscular injection of propamidine (4-4'-diamidino diphenoxy propane).

The action of the diamidines in kala-azar is of considerable interest as they are the first chemotherapeutic agents not containing antimony which have been found to have a curative action in this disease. Stilbamidine is among the most potent remedies now available for the treatment of kala-azar with the outstanding advantage that it appears to be equally effective in antimony resistant cases. At present the limiting factor in its use is toxicity. Patients receiving the drug intravenously often experience unpleasant immediate reactions which have been studied and described in considerable detail (cf. LOURIE, 1942; KIRK and HENRY 1944). We seem to have been less troubled by the immediate reactions in the Sudan than workers in other places. This is probably because we used the diamidines only for hospital in-patients, who were always in the horizontal position, in bed while the injections were being given. From the literature it would appear that treatment by the intravenous route is unsuited to the out-patient department on account of these immediate reactions and should be reserved for hospital in-patients. It should be remembered, however, that the introduction of the diamidines has opened up an indefinite number of new possibilities in therapeutics, and there may be even better compounds in the series which have still to be examined. With the introduction of the more soluble preparation of stilbamidine it may already be possible to eliminate most of the undesirable immediate reactions by giving the treatment intramuscularly instead of intravenously but we have not so far had any experience with the new compound.

For administration to human beings solutions of stilbamidine must be used immediately they are prepared as the drug is unstable in aqueous solution. This applies also to many other products used in modern chemotherapy such as neostibosan and the arspenamines, and is unlikely to be a source of trouble in the administration of the drug in proper hospital conditions. In the case of stilbamidine the products of decomposition in aqueous solution are highly toxic, but the toxic action is to some extent cumulative and does not become manifest until after a latent period.

The instability of stilbamidine in aqueous solution was not at first appreciated and this led to some unfortunate results. In 1941 stilbamidine became a general issue for the treatment of kala-azar in the Sudan partly because of

the favourable reports of its use in this disease cited above and partly because neostibosan had become unobtainable. With the more general use of the drug in the Sudan successful and often dramatic results were at first reported. But as time went on complaints were heard about various toxic effects. Some of these toxic effects were immediate and transient, similar to those which had already been observed, but others, which developed later—usually some time after the completion of treatment, with apparent cure of the disease—were more serious. Some of the patients developed nervous disturbances, others were suddenly attacked by nausea and vomiting passed into coma, and died in 1 to 4 days from the onset of symptoms.

The writer had been occupied in the meantime with matters other than kala azar, but attempts were made to investigate the reports of toxic reactions as soon as this became possible. Preliminary enquiry left no doubt about the fatalities and no alternative to the conclusion that the patients had been poisoned by the drug. It was at first exceedingly difficult to reconcile this with the results which we had obtained earlier, especially as it was found that the patients who had been treated in 1939 and 1940 were still alive and well (cf KIRK and SATI, 1943) after a period of 2½ to 3 years, in spite of the fact that many of them had received considerably larger total doses than those which had produced delayed poisoning in later cases. For a short time it looked as if we had to deal with a repetition of the circumstances of MORGENROT's discovery of the cure of experimental pneumococcus infections in mice by optochin, which failed on practical application in man (DAVE 1943) only because of an unforeseen toxic action of this compound on a proportion of human patients. However, investigations were taken up along various lines (cf KIRK and HENRY, 1944) in the hope that some other explanation might be found.

Among other things it was noted that in the early cases treated by KIRK and SATI the solution had always been freshly prepared immediately before injection whereas later cases had been treated with solution which had been prepared and stored in rubber capped bottles. It was possible to demonstrate by experiment that the latter was more toxic than freshly prepared solution. Prof. WARRINGTON YORKE who had been kept informed of the results was then able to show that the increased toxicity in old solutions was due to a specific action of light on the drug in solution and was associated with a diminution in antiparasitic activity. YORKE (1942) was able to make the further deduction that the primary change occurred in relation to the ethylenic linkage since only the unsaturated diimidines were affected by exposure to light. Thereafter it was comparatively easy to show that the late toxic effects which had been observed in the Sudan were not due to stilbamidine *per se* but to another compound which is produced rapidly in irradiated solutions of this drug and that they are avoided by the use of freshly prepared solutions (KIRK and HENRY 1944). All forms of delayed toxic action however are not avoided by



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this precaution, since the neuropathy involving the fifth nerve which was first described by NARTER and SING GUPTA (1942) occurs in cases which have been treated with freshly prepared solutions (SING GUPTA, 1943).

During those investigations our attention was directed to a factor in chemotherapy which merits more study than it has received hitherto, namely adsorption. Some chemotherapeutic agents like stilbamidine and mepracrine are characterized by strong adsorptive properties, which others, like quinine and penicillin do not possess. At present it is not possible to say whether in the former group the chemotherapeutic action is in any way related to the adsorptive properties or not. The adsorptive properties are, however intimately connected with the fate of the drug after its introduction into the human body (cf. KIRK and HENRY 1944) and, therefore with the important questions of dosage, methods of administration, and possibly also of toxicity.

#### COURSE OF THE DISEASE DURING TREATMENT AND CRITERIA OF CURE

The best evidence of cure in kala-azar is the absence of relapse over a period of several months after the termination of treatment. NARTER (1932) estimates that the absence of relapse during a period of 6 months after treatment can be taken as evidence of cure in Indian kala-azar but it is possible that in other varieties a longer period may be required.

From the clinical point of view it may sometimes be difficult to estimate when a case has had sufficient treatment and can be regarded as provisionally cured. The question is one of importance, because experience shows that cases which relapse after insufficient treatment are subsequently much more difficult to treat than primary cases. As far as we are aware, the development of induced drug resistance has not been satisfactorily demonstrated in leishmaniasis, but ADLER and TEICHERKOWITZ (1941) have found that both with the diamidines and with aromatic antimony compounds repeated injections of sub-therapeutic doses may result in an apparent stimulation of the infection. This is in accord with clinical experience in the Sudan. SATI (1942) has already pointed out that cases which are given initial small doses, gradually increased to a maximum, generally do not react so well to treatment as those which are given large doses from the beginning.

The final opinion that cure has been reached is essentially a matter of clinical judgment, since there are at present no clinical or laboratory tests which can do more than indicate the probability of successful cure at this stage. We have described elsewhere (KIRK and SATI 1940) the criteria used by us to estimate progress during treatment and to assess the probability of permanent cure. They were the decline of the fever, improvement in general condition, increase in body weight, reduction of the splenic tumour, return of the blood picture to normal and disappearance of the parasites from splenic pulp, lymph gland juice and bone marrow. Little reliance can be placed on

any one of these criteria without reference to the others, but taken together they enable a fairly accurate estimate of progress to be made.

The first effect of treatment is usually to reduce the fever, and until this occurs there is little point in applying the other tests. Sometimes a remarkable improvement in the general condition and well-being of the patient accompanies the initial decline of the fever, which may be very deceptive. Patients often consider themselves cured at this stage, and as a rule considerably longer treatment is required to bring about the desired improvement in the blood picture, negative parasitological findings, and reduction of the splenic tumour.

With regard to the blood picture, improvement in the red cell count is usually more rapid than that of the white count, but the latter has probably more prognostic value. According to NAPIER'S (1932) experience in Indian kala-azar no case relapsed in which the total white count at the end of treatment was over 6,000 per cmm, although only 25 per cent of cases below this figure did so. In the Sudan it is not uncommon to find some persistence of the leucopenia with relative lymphocytosis for a considerable period after the other tests have indicated cure. Eosinophils are generally absent in the routine counts in kala azar and their reappearance is a good prognostic sign.

Complete disappearance of the splenic tumour is sometimes difficult to obtain in long standing cases where fibrotic changes may have taken place. It is not necessary for presumption of cure. In the Sudan it has frequently been noticed in successfully treated cases that the splenic tumour, which had diminished considerably during treatment but had not disappeared, continued to shrink after discharge from hospital until 3 to 6 months later it had disappeared without further treatment. On the other hand, complete disappearance of the splenic tumour during treatment is no guarantee of cure, cases in which this occurred have been known to relapse, although this is uncommon. We should hesitate to regard any case as a presumptive cure in which no reduction of the size of the spleen had occurred during treatment. Chronic cases, with large, very hard spleens are sometimes encountered in which the size of the spleen does not alter during treatment, although the other tests indicate great improvement. The ultimate prognosis in such cases is not good.

Even the disappearance of parasites from spleen, bone marrow and lymph glands may not be an entirely reliable criterion of cure. This is partly because the usual method of examining smears under the microscope is not infallible and may fail to detect parasites when they are still present in small numbers. Nevertheless, cases have been observed in which relapse occurred even although cultural methods had failed to demonstrate parasites at the end of treatment. On the other hand, NAPIER (1932) records that cases of Indian kala azar, in which cultures showed that viable parasites were still present at the end of

treatment, continued to improve after discharge, and made a complete and permanent recovery without any further treatment. In spite of these observations we have made it a rule not to regard a patient as provisionally cured until several consecutive examinations have failed to reveal parasites in the splenic pulp and gland juice, and a final test shows that they are absent also from the bone marrow. The persistence of parasites in any dermal lesions which have appeared during treatment is different. We regard this as perfectly compatible with the conclusion that cure of the visceral disease has been achieved.

We have not found it possible to lay down a standard course of treatment after which it can reasonably be presumed that most patients will be cured. So far the only type of the disease in which this has been successfully accomplished is the Indian one. This may be because of the more extensive clinical experience which has been accumulated in India, but it may also be because the Indian disease is more uniformly amenable to treatment than other forms. ADLER and TONENWORMETZ (1939) have shown that in hamsters infected with kala-azar the total amount of stilbamidine required to eradicate the infection depends on the intensity of the infection. ASCHMANN (1923) reached a similar conclusion about tartar emetic in human beings. Our own observations show clearly that the amount of treatment required for cure varies within wide limits in different cases. Factors influencing the amount of treatment required for cure are the strain of the parasite, stage, type and intensity of the infection, tolerance of the drug and the presence of severe complications and intercurrent infections.

#### POST KALA-AZAR DERMAL LEISHMANIASIS.

KIRK and DREW (1938) described various skin eruptions which appear in a proportion of cases of Sudan kala-azar at the end of treatment. Further studies of this interesting phenomenon were reported in previous papers of the present series (KIRK and SATTI 1940b; KIRK 1947), which should be consulted for the details. We have suggested that these skin eruptions are in many ways similar to the post-kala-azar dermal leishmaniasis of India, although their relation in time to the recovery from kala-azar is slightly different. Our experience indicates that the development of a typical post-kala-azar dermal eruption during or after treatment has a good prognostic significance. Many cases undoubtedly attain complete and permanent cure without dermal manifestations, but from experience we have come to regard the development of a typical post-kala-azar dermal eruption in any case as a valuable addition to the other criteria of cure.

It is of great interest to note that similar skin eruptions were observed in successfully treated cases of Sudan kala-azar many years ago, although their

significance was not then appreciated. Recording leucoderma as an unusual toxic effect of antimony, CHRISTOPHERSON (1921) described and illustrated what we should now regard as a typical example of nodular depigmented post-kala-azar dermal leishmaniasis (cf. illustrations in KIRK and MACDONALD, 1940).

### THE MECHANISM OF CURE IN KALA AZAR

The manner in which drugs act to bring about cure in kala-azar is still imperfectly understood, but the known facts relating to post-kala-azar dermal infection indicate that the subject is one of great interest. Anti-kala-azar drugs have not received the same intensive research as has been devoted, for example, to the arsphenamines and sulphonamides. It is not yet possible to describe their action in terms of precise biochemical knowledge such as is now available in the case of the sulphonamides and, to a lesser degree of the arsenicals. The views here put forward are based entirely on clinical observations, and can only be stated in general or descriptive terms.

Recovery from kala-azar does not necessarily imply complete elimination of the parasites, or *therapia sterilans*, although no doubt this is achieved in a number of cases. There is evidence to suggest that it occurs most commonly in acute epidemic forms of kala-azar. In the ordinary endemic form, however, it has been shown that complete and permanent cure of the visceral disease is often compatible with an extensive invasion of the skin by the parasites when they may produce cutaneous lesions after a latent period of several months in Indian kala-azar or in the Sudan disease towards the end of treatment. This residual or post-kala-azar infection has to be differentiated clearly from incomplete cure. In the latter relapse of the visceral disease is likely to occur whereas all the evidence indicates that visceral relapse is exceedingly rare once the dermal infection has become established. There is also evidence that post-kala-azar dermal leishmaniasis is associated with immunity to reinfection. The condition has been studied most extensively in treated cases, but it has also been observed in rare cases of apparently spontaneous recovery from the visceral disease (ACTON and NAPIER, 1927), thus suggesting that it is associated with an immunity response of some kind. Anyone interested in analogies can hardly fail to notice the close parallelism between this condition and verruga peruviana another *Phlebotomus* transmitted infection.

EHRICH's original conception of the action of chemotherapeutic agents was that of "magic bullets"—selective poisons killing only the parasites by virtue of a highly specific affinity for certain chemical groups or constituents of the cells attacked. In kala-azar something more than this is required to explain the ascertained facts. BOYD, NAPIER and ROY (1931) have pointed out that the therapeutically effective pentavalent compounds of antimony have no action whatever on cultural forms of leishmania in concentrations ten times

greater than they could ever be present in the blood when given in therapeutic doses. In the human subject cure appears to be associated with the disappearance of the parasites from the visceral reticulo-endothelial tissues; it does not necessarily include their elimination from the skin but seems often to be associated with an extensive invasion of this tissue. The suggestion that the drugs used in treatment may not reach the skin, in sufficient concentration to affect the parasites there is not an adequate explanation of this phenomenon. Little is known about the distribution of the diamidines in the body but as regards antimony Boyd NAPIER and ROT (1931) found appreciable quantities of this drug in the hair and traces are excreted in the sweat (CROPPA, 1936). It may be noted also that once the dermal infection becomes properly established there appears to be little or no tendency to visceral relapse. Even after long intervals without further treatment, which would allow complete elimination of the drug from the visceral tissues the parasites do not again invade the internal organs. Apparently some profound and lasting change in host-parasite relationship occurs during treatment. Although the parasites may not be completely eliminated from the body the course of evolution of the infection becomes, in fact, similar to that observed in spontaneous recovery. From our observations in the Sudan it can be stated that this is so in cases treated with the diamidines as well as in cases treated with antimony—a point of some interest, since the active groups in the diamidines and in the antimonials are entirely different from each other in chemical constitution.

#### DISPOSAL OF TREATED CASES.

From the practical point of view a patient with post-kala-azar dermal leishmaniasis is little different from a carrier. The lesions may be very inconspicuous, yet persist for a very long time—over 20 years in a case described by the writer (KIRK, 1942). General health is unaffected, and there seems little tendency to relapse of the visceral disease. But, owing to the situation of the infection in the skin, sandflies can readily take up the infection from such an individual. In places where suitable ectoparasites are abundant he may infect large numbers of them, and thus indirectly be a source of danger to other people. Some of the small, isolated outbreaks of kala-azar which have occurred among troops in the Sudan (KIRK, 1939), and more recently in East Africa (COLE *et al.* 1942) may have been brought about in this way.

NAPIER (1935) has suggested that only a small number of patients develop skin infections after recovery from the visceral disease. After treatment, exhibit clinically recognizable skin lesions. On the other hand, a carrier condition, in which the skin is not infected, yet infective for sandflies. The final disposal of patients with kala-azar should therefore be a matter of the

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with the hygiene of troops in barracks and other places where known or suspected vectors are prevalent

The sanitarian who proposes to use mass treatment as a means of reducing the incidence of kala-azar in the endemic areas must also take account of post-kala-azar dermal leishmaniasis. From his point of view it is an unfortunate coincidence that this disease, for which the available methods of treatment are among the most perfect in medicine, should have such a peculiar course of evolution in the human body.

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# BILHARZIASIS IN THE GEZIRA IRRIGATED AREA OF THE SUDAN

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Bilharziasis, *S haematobium* or *S mansoni*, is endemic throughout most of the Sudan, both forms being found north of parallel 12 N, principally *mansoni* south of parallel 8 N, while in between is an area which is apparently bilharzia free

It is well known that bilharzial infection is one of the great dangers in any perennial irrigation scheme, and this was fully recognized as regards the Gezira scheme from its inception

This scheme covers an area west of the Blue Nile taking its water from the reservoir formed by the dam at Sennar. From its origin there, the main canal runs a course of some 130 miles roughly parallel to the river and waters a total area of some 850,000 acres. Its development, roughly from south to north is shown in Table I

TABLE I  
DEVELOPMENT OF THE GEZIRA IRRIGATION SCHEME.

Year	Area irrigated (acres)	Year	Area irrigated (acres)
1925	240,000	1933	700,000
1928	380,000	1936	800,000
1929	510,000	1938	850 000

Since 1938 there have been minor extensions only but future developments may bring the area under irrigation up to nearly double its present acreage

### *System of Irrigation and Cultivation.*

Irrigation is by high level canals, and the system comprises the following lengths of channel, constructed maintained and operated by Government —

Main canal and major distributaries 600 miles.

Minor distributaries 2,000 miles.

Water enters the main canal from the reservoir on about 15th July each year and full supply is reached by the end of the month. The canal is normally closed to irrigation early in April, but this is sometimes postponed until early May. During this period of closure supply has to be maintained in many canals for village and house water supply the remainder of the minor distributaries being dried out.

Before the reservoir is emptied, all minor distributaries on summer water supply are filled to their highest level, and the supply is maintained by means of a pump, which delivers water from the river into the main canal some 50 miles from the dam. Thus from early May until mid-July the water is practically stagnant throughout the main and major canals, and in those minor distributaries on summer water supply.

All watering of crops is done by day and this entails storage of water in minor distributaries by night. The watering rotation in field channels varies from 10 days in October to 16 days from January on, but whatever the period, half of it is taken up with watering, the field channels being left to dry during the other half.

Cultivation is done on a 4 year rotation a quarter of the area being under the main crop cotton, each year one eighth under millet and one eighth under a bean crop, the remainder being fallow. Supervision of cotton cultivation is exercised by inspectors of the concession companies, who also supervise field water control.

Abdel Magid area, of some 36,000 acres is an exception. It is run under Government supervision, and watering is done throughout the 24 hours, and there is, therefore, no storage of water in the minor distributaries, though the field watering rotation is the same as in the rest of the scheme.

### ADMINISTRATION AND POPULATION

For administrative and medical purposes the area is divided into two districts, Northern and Southern, and the population was estimated in 1944 to be: Southern District 349,000 Northern District 1,000.

Figures for the early years are not available but there has been an estimated increase of 100,000 during the past 8 years and it is probable that the population did not exceed half its present figure before 1925. The increase is partly due to the effect of the greater prosperity and higher standard of living, but is also in large part due to immigrants, mainly from the West having settled in the area.

In addition there is a yearly influx of immigrants during the cotton picking season from December to March. This force has increased from about 40,000 in the early years to some 90,000 annually at the present time, of which 36 per cent come from the White Nile District, 18 per cent from the Sudan east of the Blue Nile, and the remaining 46 per cent from Western Sudan and British and French West Africa.

There are some fifty dispensaries scattered throughout the area, half of which are based on the hospital in Wad Medani, the Province headquarters, and the other half on the hospital in Abu Usher in the Northern District. Each of these is in the charge of a medical assistant, who has a microscope and is trained to examine urine and faeces for bilharzia eggs.

### Historical

Before the area was first irrigated the population was confined to villages along the river, which is swift flowing and subject to a large annual rise and fall, and to scattered villages inland, which got their water from wells and, as stated by HUMPHREYS (1932) bilharziasis was practically absent. Nevertheless it was realized that this would be a great danger in the irrigation scheme. The Director of Medical Service wrote in the *Annual Report for 1925* —

"The most urgent sanitary problem confronting the Sudan Government at present is that of preventing the Gezira canals becoming infected with bilharzia. If this were to occur the result would be disastrous and probably irretrievable. There are certain endemic areas in the Sudan where the population is heavily infected. It is impossible by any system of quarantine to prevent natives of these areas reaching the canalized area and therefore we can only deal with this danger by —

- 1 Carrying out an active campaign in all endemic areas
- 2 Constant examination of all Sudanese working parties
- 3 Immediate examination of all immigrants by Sanitary Hakims (Medical Assistants) in the dispensaries

As action under (2) and (3) is very uncertain (1) is the most important."

It was also pointed out that if bilharziasis became endemic in this area the probable eventual result would be a rate of infection comparable to that found in the Delta in Egypt with the added complication that malaria is endemic in this area whereas it is not found in the Delta and that therefore the effect on the health of the population would be so much worse.

By 1927 it was apparent that bilharzian snails were establishing themselves in the canals. *Bulinus* species more rapidly than *Planorbis*, and a few locally contracted cases of *S. haematobium* were being found.

In the *Annual Report for that year* the Director of Medical Service wrote

"It would seem that the bilharzia position in the irrigated area is grave but is not yet desperate. If however quarantine measures against Westerners and Egyptian labourers is relaxed if regulations in the Gezira are not enforced if anti-bilharzial measures in the other endemic areas are not carried out and bilharzia is allowed to spread then the irrigated area will sooner or later, become infected and once generally infected, unless some new and far more effective means of dealing with the disease is discovered it will be impossible to eradicate this disease. How serious the consequences of this infection would be under the climatic conditions of the Gezira has already been pointed out."

6. Provision of latrines. In 1932 it was proposed (*Sudan Medical Service Annual Report*) that in view of the density of the population, and the proximity of the canals, the provision of an auger bore latrine for each separate hut must be considered an essential measure of prophylaxis. These were tried but were found to be unsatisfactory and deep pit public latrines were made in some of those villages which were sited near to canals. These are used by many of the villagers, but apart from there not being adequate provision in any one village it is doubtful if all would use them were such provision made.

7. Disinfection and drying out of canals. It was originally hoped that the majority of the minor distributaries would be dried out for the whole period of the closure, and would therefore be dry for over 2 months, and it was proposed that any that could not be dried should be disinfected but it soon became apparent that this could not be done.

As the scheme extended so did the demand for canals to be on summer water supply grow until now between one-half and two-thirds of the mileage of minor distributaries is dried out each year and most of those on which villages are sited are not dried. Also water is kept in the majority of those that are dried until the end of May and they are then dried off slowly.

BARLOW (1933) first pointed out that *Planorbis* and *Bulinus* species could withstand long period of drying, and this has since been confirmed by others, and this is particularly so if slow drying of canals enables them to bury themselves in cracks in the ground. Therefore though it is possible that such drying kills some if not all, of the infected snail it is probable that it has little effect on the uninfected ones.

It was found impossible to disinfect all those canals which were not dried, and disinfection is now limited to those on which are sited villages selected for complete examination. It is done with sizolin or Prince Regent disinfectant. The results of drying and disinfection are discussed later.

8. Widespread propaganda and strict regulation against entering canal. Propaganda is not effective in such a community and it has been found quite impossible to enforce the regulations.

## OBSERVATIONS AND INVESTIGATIONS, 1942 TO 1945

In regard to these observations two important points should be noted. First, that the author was not in a position to make detailed scientific surveys, as he had to make use of normal medical staff and facilities and, as such work had to be fitted in with all his other manifold duties, he was not able to give personal attention to details of the work. Secondly, that although the observations have been confined to the northern half of the irrigated area, there can be no doubt but that the findings apply equally to the whole area. In fact, the position in the southern half is probably worse as it has been irrigated for a longer period.

It was first considered necessary to review the whole position, and the following points became apparent —

1 That within a few years of the opening of any part of the irrigation system bilharzial snails established themselves in the canals and increased in numbers rapidly.

2 That *Bulinus* species, for some reason unknown, established themselves more quickly than did *Planorbis* species, but that by 1942 both species could be found in large numbers throughout the canal system.

3 That the weed growth, both of permanent grasses and of floating weeds, was excessive in the canal system, particularly in the minor distributaries, and that these conditions made the canals ideal places for the snails to live and breed in.

4 That each year there was an influx into the area of people, among whom there was a high rate of infection with bilharzia. An average of some 40,000 Westerners came in each year, among whom 17.5 per cent were carriers of *S. haematobium*, and a similar proportion were probably carriers of *S. mansoni*. Some 30,000 people came from the White Nile area, among whom 60 per cent to 90 per cent are carriers of either *S. haematobium* or *S. mansoni*.

5 That the minor distributaries are almost universally used by the indigenous and immigrant population for domestic water supply purposes, and that not only do the people bathe in these canals but they also perform their natural functions, either in the water or on the edges of the canals which are subject to nightly flooding.

6 That the minor distributaries are the main source of local bilharzial infection although some few villages do use major canals for their domestic water supply, and it is known that there are infected snails in some of these. The field channels are not considered an important source of infection as they are not used for bathing and other purposes, and any particular channel only carries water in 3 out of every 8 years. They are also kept fairly free of weeds and are baled dry between each watering. Infection in the fields is improbable as the people only work in them when they are dry.

It was then decided that three points required investigation. First, to discover if the incidence of *S. haematobium* infestation among the indigenous population was higher than had previously been supposed.

Secondly to investigate the incidence of *S. mansoni* infection among the indigenous population.

TABLE IV  
RESULTS OF VILLAGE EXAMINATIONS FOR *S. haematobium*.

Year	Village.	Adults.			Children.			Total		
		Number		Per cent	Number		Per cent	Number		Per cent
		Examined.	Infected.		Examined.	Infected.		Examined.	Infected.	
1942		423	94	22	293	117	40	716	211	29
	b	143	148	58	173	134	78	425	282	66
		147	81	55	87	71	82	234	152	65
	d	142	61	43	106	59	55	248	120	48
		112	38	34	74	39	52	186	77	42
1944	f	84	6	7	63	11	18	151	17	11
	g	25	17	70	49	22	45	124	49	39
	h	23	1	4	20	14	70	43	15	35
		360	18	5	48	43	13	608	61	10
	j	183	40	22	63	38	59	246	78	31
1945	b (3)	287	31	11	169	72	42	456	103	23
	(2)	186	22	12	124	65	52	310	87	28
	k	253	33	13	182	132	73	435	165	38
	l	375	29	8	235	63	27	610	92	15
	m	183	4	2	87	17	20	270	1	0
		70	18	26	34	8	24	104	23	22
	p	94	1	1	54	2	4	148	3	2
	(3)	864	100	12	408	166	41	972	266	27
Total, excluding re-examinations		2,230	617	28	1,614	819	51	4,772	1,436	30

Thirdly to investigate more closely the infestation of the canals by bilharzial snails

#### INCIDENCE OF *S. haematobium*

From a study of the records it was seen that cases of *S. haematobium* infection were reported from many villages scattered all over the area, and it was decided to select a few of these villages each year for examination of the whole population. These examinations had to be done by the local medical assistants and so only villages in the area of the most reliable of these were

selected The number examined each year was further limited as it was as much as one man could do to examine one large or two small villages. Examinations were done in 1942, 1944 and 1945. For various reasons, it was not found possible to carry out any examinations in 1943. The results are given in Table IV.

It should be noted that it was, in practice, found impossible to examine the whole population of these villages as there were always some absentees, but from 70 per cent to 90 per cent were examined in each case, always excluding children under 2 years of age and those of very advanced years.

It will be seen that, excluding villages which were re-examined, the average rate of infection was 21 per cent in adults and 45 per cent in children, with an overall average of 30 per cent with extremes of 2 per cent and 66 per cent.

It is difficult to explain why there should be such a marked difference between these figures and those previously obtained by the annual survey, the highest of which were 0.77 per cent in adults and 1.5 per cent in children, but it is considered that the former throw grave doubts on the validity of the latter.

It has been found that, in the routine examination of urines in dispensaries, 231 (61 per cent) out of 376 cases found in 1942 were locally contracted, 293 (81 per cent) out of 362 in 1943, and 180 (59 per cent) out of 307 in 1944. Results of the village examinations are not included in these figures. This shows that over a period of 3 years well over half of the cases so found are contracted locally.

In these examinations the percentage found infected amongst immigrants does not exceed that for the indigenous population. Comparative figures for 1944 are given in Table V.

TABLE V  
COMPARATIVE FIGURES FOR ROUTINE EXAMINATIONS OF URINES IN DISPENSARIES FOR 1944

	Adults			Children		
	Number		Per cent	Number		Per cent
	Examined	Infected		Examined	Infected	
Indigenous	15 683	176	1.12	9,280	432	4.40
Immigrant	10 077	109	1.09	4,017	18	0.45

The figures for the indigenous population include village examinations

We know that the rate of infection among Westerners, who form the bulk of the immigrants examined, is at least 17.5 per cent, and, therefore, it is



reasonable to assume that the general rate of infection amongst the indigenous population throughout the area is much nearer to the figures found in these village examinations than to those found by the annual survey as done previously.

One further point deserves notice, and that is the difference between the figures for adults and those for children. It will be noted that, in some cases, the percentage among children is from four to five times as great as it is among adults, while in one case it is ten times as great. It will also be seen that in those villages with a very high rate of infection the figures more closely approach one another.

One obvious factor in this is, that because of the chronicity of the disease more cases are likely to be missed by a single urinary examination in adults than in children, and this is also likely to be true where there is a low rate of individual infestation.

The other factor is immunity and from the results found here, it is suggested that where the concentration of cercariae and hence the intensity of individual infection is low the immunity acquired by the time adult age is reached is sufficient to prevent a high rate of re-infection, while, as the concentration of cercariae rises, so does the acquired immunity become less and less able to combat the higher intensity of individual infection.

#### INCIDENCE OF *S. mansoni*

This is much more difficult to determine than that of *S. haematobium* for not only is there considerable difficulty in getting the people to consent to faecal examination but the results of surveys done by a single faecal examination are not reliable. It has been pointed out by Scott (1937a) that no one method will detect all the positive cases, and this when he was using concentration methods, and more recently ORTOLINA and ATENCIO (1943) have concluded that direct faecal examinations fail to diagnose eleven to eighteen out of every twenty cases.

In spite of these difficulties examinations have been carried out in a few villages and up to 5 per cent. have been found infected. In one village where *mansoni* infection is known to occur no eggs were found in the stools, but three cases were found to have *mansoni* eggs in the urine. Therefore some other means must be sought to form some estimate of the incidence of this form. Until and including 1940 the number of cases of *S. mansoni* recorded in the Northern District varied from nil to ten, and there is no record that any of these were locally contracted. The figures for the years 1941 to 1944 are shown in Table VI.

Medical assistants in dispensaries also do routine examination of stools, and the figures for such examinations among the indigenous population in 1944 are shown in Table VII.

TABLE VI  
*S. mansoni* IN THE NORTHERN DISTRICT, 1941-44

Year	Cases	Deaths	Number of cases locally contracted	Per cent
1941	32	6	17	53
1942	148	5	64	43
1943	183	7	73	40
1944	130	4	67	51

TABLE VII  
 EXAMINATION OF STOOLS OF INDIGENOUS POPULATION IN DISPENSARIES, 1944

Adults			Children			Total		
Number		Per cent	Number		Per cent	Number		Per cent
Examined	Infected		Examined	Infected		Examined	Infected	
3 596	44	1 22	1,462	23	1 57	5,058	67	1 32

It will be noted that the percentage found infected is comparable to that for *S. haematobium*

From the records of the cases coming under notice, it is considered that there is a high rate of infection in at least eleven villages, which are situated on different and widely separated canals, and there are known to be infected snails in at least fifteen canals, and there is strong presumptive evidence that they are infected in many more

Therefore, although there is no certain knowledge as to the actual general rate of infection with *S. mansoni*, it can be definitely stated that it occurs in the area, that it is probably widespread, and that in certain places there is a fairly high rate of infection

#### SNAIL DISTRIBUTION

No detailed observations on snail distribution, or on seasonal variations have been possible, but snail collections have been made in minor distributaries scattered throughout the area, and at various times of year Two methods have been used —

1 A standard bundle of palm fronds is placed in the water in the middle

of the canal and left for 24 hours, at the end of which time the number of snails on it are counted.

2. A four gallon petrol tin from which two sides have been cut, is placed in the weeds at the edge of the canal. The weeds and mud from inside the tin are removed, placed in a dish and the snails counted.

By the first method counts up to seventy-five *Bulinus* and thirty-five *Planorbis* have been found, and by the second method up to sixty *Bulinus* and thirty *Planorbis*.

Some thirty minor canals have been kept under continuous observation for a whole watering season, from August, 1944 to March, 1945 counts being made at from two to four places on each canal twice a month. No definite conclusions can be drawn from observations extending over only one season, but there would appear to be very little correlation between drying out and the appearance of snails in the early months, as they were found in August in some canals which had been dried, and, on the other hand, were not found in the early months in some canals which had not been dried. As would be expected, smaller counts have been constantly found in those canals where the weed growth has been lightest. The only time at which young snails have been found in any numbers has been towards the end of the period of observation. Considerable variations in counts from different canals, and from the same canals at different times have been found, but the only conclusion that can be drawn from the observations is that infestation with both *Bulinus* and *Planorbis* is heavy throughout the area.

As has been pointed out above, there is one part of the area—the Abdel Magad scheme—where night watering is practised and, therefore, not only is there a constant flow of water through the whole system, including the minor distributaries, but these latter are narrower and more steep sided than those in the main part of the scheme and the growth of permanent weeds is considerably less.

Comparative snail counts have been made in minor canals on night storage and night watering systems. By the petrol tin method from two to ten times as many bilharzial snails were found at the edges of the former as in the latter while by the palm frond method from two to four times as many are found. At the tails of the canals the numbers found were approximately equal in both systems.

The deduction can be made that the number of resident snails is up to ten times as great in the night storage canal as it is in those on night watering, and that in the latter they tend to get washed through the canal to the tail and out on to the fields.

#### THE DISINFECTION OF CANALS.

Observations were made to try to determine the value of disinfection of isolated canals with Prince Regent disinfectant as an anti-bilharzia measure.

In 1942 examination for *S. haematobium* was done in three villages (*a*, *b* and *c* in Table IV, p 486), and the results were —

- a* Adults 22.2 per cent, children 39.9 per cent
- b* Adults 58.4 per cent, children 77.9 per cent
- c* Adults 55.1 per cent, children 81.6 per cent

All cases found were treated with tartar emetic, and the canals supplying the villages were disinfected at about the time treatment of all cases was complete

Villages *b* and *c* were re-examined in 1944 with the following results —

- b* Adults 10.8 per cent, children 43.8 per cent.
- c* Adults 11.6 per cent, children 52.4 per cent.

The canal supplying Village *a*, was also disinfected in 1943 and 1944, and when re-examined in 1945 gave the following figures —

Adults 17.7 per cent, children 41.3 per cent

From these cases it would seem that a high rate of infection can reappear in a village within a short time of combined disinfection of the canal and treatment of all discoverable cases

The reason for this is that the cycle of infection is kept going because —

- 1 It is impossible to synchronize elimination of snails by disinfection and elimination of human infection by treatment
  - 2 Cases are bound to be missed in an examination of a village
  - 3 Infected persons from outside come to the village or use the canal
  - 4 A number of the cases treated relapse
- KHALIL and AZIM (1938a) found that the relapse rate after treatment with tartar emetic, in a place where re-infection could be excluded, was 35 per cent

### DISCUSSION

From the observations that have been made during the past 3 years it can be concluded that —

- 1 The incidence of *S. haematobium* infection is fairly high throughout the area
- 2 Infection with *S. mansoni* is widespread, and the incidence is probably fairly high in certain areas
- 3 The rise in incidence of these diseases has probably been gradual over a period of some years
- 4 The measures taken heretofore to prevent bilharziasis becoming endemic in the Gezira have failed to achieve that object

Further, it is surmised that the rise in incidence is likely to be increasingly rapid in the near future. It has been shown by KHALIL and AZIM (1938b) that the infection rate with *S. haematobium* increased in villages in Egypt, in one case from 10 per cent to 40 per cent, and in another from 2 per cent to 75 per cent in the space of 3 years

The effect on the general health of the population has not been very noticeable up to the present, but the observation of KILMER (1934) on school children are an indication that, though infection may pass unnoticed and cause no inconvenience to the patient, it nevertheless has considerable effect on his physical and mental powers. Also, as GILFAND (1942) points out, it predisposes to avitaminosis, tuberculosis, pneumonia and many other diseases. It would appear probable that, should the incidence in the future approach that found in the Delta in Egypt the result on the health of the population might well be disastrous.

It is, therefore, essential that measures should be taken immediately with a view to stopping any further increase, and to eventually reducing the incidence to a minimum.

SCOTT (1940) and BRUNST (1941) in Venezuela, and SCOTT (1942) in Egypt have pointed out that sanitary measures are difficult, expensive and ineffective, and that the only hopeful measure of prevention is the reduction of the number of snails and the truth of this has again been demonstrated here, for most of the measures used have been directed toward prevention of infection of canals by carriers.

It has been found impossible to keep the people from using the canals for bathing and other purposes, and even if every village in the area were removed as far as possible away from the canals, and all supplied with wells and adequate latrine accommodation—measures which are ruled out on the ground of expense—the people would still use the canals, and it is doubtful if the incidence of bilharzias could be kept to its present level, let alone reduced.

Therefore although it is necessary to continue and extend all the sanitary measures, steps must be taken to reduce the number of bilharzial snails in the canals to an absolute minimum.

It is impossible to eliminate snail from the canals entirely as fresh ones are continually being brought into the system from the river and the problem, therefore, resolves itself into making the conditions in the canals such that they will not be congenial places for the snails to live and breed in.

The first step toward achieving this must be a great reduction in the weed growth in the canals, particularly and primarily in the minor distributaries. In many of these the growth of permanent grasses is so excessive that it extends practically across the whole width of the canal, and it is only for a short period after clearance has been done for irrigation purposes that these canals are relatively free of permanent weeds.

From the experience already gained in the Abdel Magid system, it is known that the weed growth in the canals of a 24-hour watering system is considerably less than in canals that are used for night storage the reason being that the canals are of hydraulic section which is deeper, narrower and steeper sided than the wide, shallow section necessitated by night storage and because

the flow of water through them is constant, and it is, therefore, suggested that one method of effecting a reduction in weed growth would be to change the whole system from night storage to 24 hour watering. This would also enable the number of minor canals to be reduced by about one third.

Were this done, weeding would still be necessary as conditions in the canals on 24-hour watering are by no means ideal and snails can live and breed there, but the work entailed would be very much less than under the present system.

Unfortunately, it would appear that the agriculturalists are very much opposed to such a change, as they fear that it would entail deterioration of the land, owing to lack of control of watering. So, if there are reasonable grounds for this fear, the engineers must be looked to for some other solution to this problem, for it is essential that the weed growth should be reduced to an absolute minimum.

Nevertheless, whatever method is used to reduce weed growth in canals, it is impossible to keep them absolutely weed-free, and SCOTT has pointed out that even where weeds are cleared, snails may still be abundant, feeding on algae on the sides and bottom of canals. Therefore, in addition to such measures, snail clearance, as has been tried in Egypt and reported on by BARLOW (1937), may have to be used. Weed clearance and snail clearance can go hand in hand.

These measures are urgently necessary, but at the same time there should be a complete ecological study of the snails so that the value of these and other measures can be assessed and others recommended.

It is also necessary that similar anti bilharzia measures should be taken in other endemic areas, and a greater effort must be made to discover and treat cases in the Gezira and other places for, though treatment of cases by itself is ineffective in controlling bilharzia yet, together with adequate measures of snail control, it will help to reduce the incidence more quickly.

In conclusion, the importance of anti-bilharzia measures to the health of the population of the Sudan must be stressed, for not only is the Gezira Irrigation Scheme and its future extensions concerned but all the other schemes throughout the country, and the large number that will probably be developed in many parts of the country in the near future.

In regard to Egypt, SCOTT has asked the very pertinent question as to whether the improvement in economic conditions gained by the use of perennial irrigation warrants the impairment of the health of the population. There can be no doubt but that the wise use of irrigation in the Sudan will be of great benefit in raising the standard of living of the people, but it is not considered that the wise use of irrigation includes exposing the people to the risk of a high rate of infection with bilharzia.

Thus, even more than in 1925, bilharziasis remains one of the most important public health problems that faces the Sudan, and in order to prevent

grave impairment of the health of the people over wide areas of the country it is essential that more effective measures be taken to reduce and control the incidence of this disease.

#### SUMMARY

1 The position as regards bilharziasis infection in the irrigated area has deteriorated considerably.

2 Infection rate with *S. haematobium* is probably at least 20 per cent. in adults and 45 per cent. in children throughout the area, with a much higher rate in certain places.

3 Infection rate with *S. mansoni* is impossible to estimate, but it is known that the infection is widespread, and that there is a fairly high rate in certain widely scattered places.

4 All measures heretofore taken have failed to control spread of these diseases.

5 Future measures must be directed against the snail population in the canals, and the first step must be a great reduction of the weed growth. These and other measures are discussed.

6 An ecological study of bilharziasis snails in this and other endemic areas in the country is necessary in order to evaluate future measures of control and to recommend others.

7 Bilharziasis is one of the important public health problems facing the Sudan, and its importance is even greater than in the past because there is likely to be considerable extension of the use of irrigation in the near future.

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*Sudan Medical Service Annual Reports* (1925-1942)

OBSERVATIONS ON *LEPTOMONAS CTENOCEPHALI*  
(FANTHAM, 1912)

BY

ALFRED J GIBBS \*

The parasite was discovered in the dog flea, *Ctenocephalus canis*, by BASILE (1910), who believed he was dealing with a species of *Leishmania*. FANTHAM (1912) placed it in genus *Herpetomonas*, and provisionally gave it the specific name *Ctenocephali*. Since, however, crithidia and trypanosomes are not found at any stage, it was later transferred to the genus *Leptomonas*.

DEVELOPMENT IN LARVA

Infection of the flea takes place during the larval stage, and is the result, as will be shown, of the ingestion of resistant bodies of leishmania form which are present in the faeces of infected fleas. The faeces consists chiefly of partially digested blood which can be dissolved in saline under a cover-glass for the study of the bodies in the living state. They are spherical and without apparent internal detail. When fixed and stained by Leishman's method they are ovoid in form and measure some  $3\ \mu$  by  $2.2\ \mu$ . The nucleus and kinetoplast are situated near one end while the rhizoplast extends from the region of the kinetoplast to the opposite end of the body. No cyst wall can be seen either when stained or examined in the fresh state in saline (Fig 1, p 497).

Larvae feed readily on the faeces of the flea, which, when dry, is found in the form of granules on the body of the dog. Newly hatched specimens, which have been experimentally fed on the faecal blood of infected fleas, are often found to harbour leptomonads on the day following hatching. Multiplication does not take place in the pre flagellate form as the infective bodies develop into flagellates. At first the parasites are confined to the mid-gut (stomach) and are very few in number, probably representing individuals which have developed directly from the infective bodies. These earliest found forms are highly motile leptomonads about  $21\ \mu$  long with flagella of the same length. There are usually one or more twists in the body (Fig 2). A constant characteristic of the larger leptomonad forms is that the nucleus is located within the anterior third of the body, and the nucleus and kinetoplast are situated in close proximity. Multiplication takes place and the

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Larvae feed readily on the faeces of the flea, which, when dry, is found in the form of granules on the body of the dog. Newly hatched specimens which have been experimentally fed on the faecal blood of infected fleas, are often found to harbour leptomonads on the day following hatching. Multiplication does not take place in the pre flagellate form as the infective bodies develop into flagellates. At first the parasites are confined to the mid-gut (stomach) and are very few in number, probably representing individuals which have developed directly from the infective bodies. These earliest found forms are highly motile leptomonads about  $21\ \mu$  long with flagella of the same length. There are usually one or more twists in the body (Fig 2). A constant characteristic of the larger leptomonad forms is that the nucleus is located within the anterior third of the body, and the nucleus and kinetoplast are situated in close proximity. Multiplication takes place and the

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mid-gut of the larva becomes heavily infected with long, slender leptomonads. Later the length of the flagellum diminishes to about one quarter of the body-length and its power of movement is reduced to a series of periodic jerks with little or no travel. At a still later stage the flagellum practically disappears, but the body remains elongated and twisted. (Fig. 3)

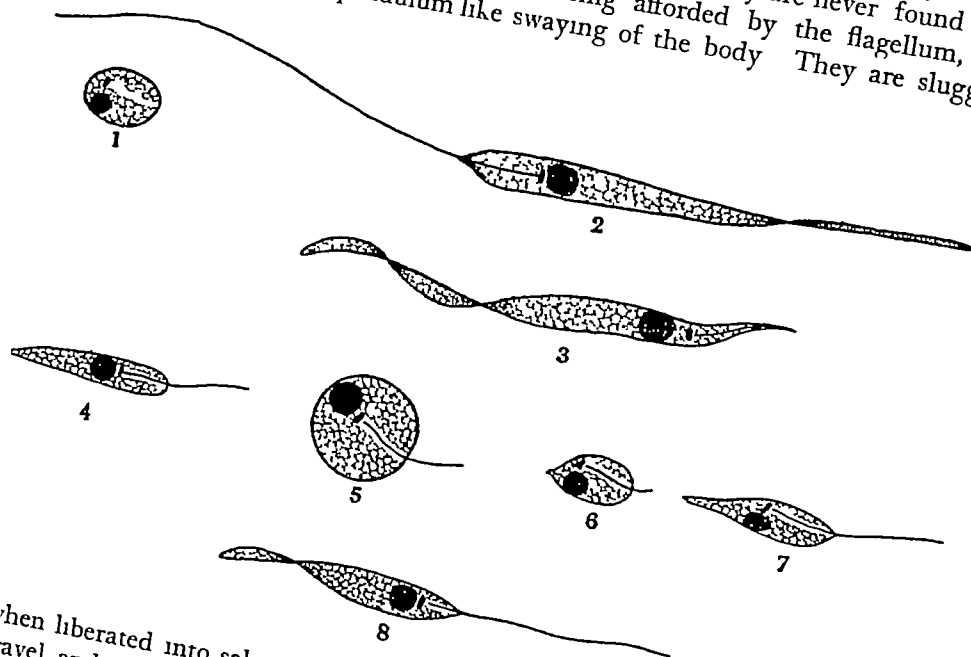
Under natural conditions larvae attain the pupal stage in about 4 days, but under the laboratory conditions available they failed to progress beyond the first moult. It is possible, therefore, that subsequent phases of the life-cycle of the parasite and its disposition in the host, although described as occurring in the larva, may normally take place after the pupal stage is attained.

About seven days after hatching the infection travels to the hind-gut which first harbours a few active leptomonads similar to those first found in the mid-gut. The body is twisted and the flagellum is long and active, but is always attached near its tip to the gut wall. Free forms are not found in the hind-gut at any time. This adherent condition of the flagellate has never been observed among leptomonads in the mid-gut. Although the mid-gut and hind-gut are occasionally infected concurrently (the former with leptomonads which have practically lost their flagella, and the latter with a few adherent leptomonads with long flagella), it is usual to find that the parasites in the mid-gut disappear when the hind-gut becomes infected. The adherent flagellates soon become rounded and take on leishmania-like form. Multiplication then becomes rapid and isolated clusters of leishmanial parasites can be found at various points in the hind-gut, but they are never found in the rectum. Later the hind-gut becomes packed with parasites. The Malpighian tubules of larvae are never found to be infected.

#### FORMS FOUND IN ADULT FLEA

As the larvae failed to attain the pupal stage, investigation has been limited to the examination of the larva and the adult flea. Parasites are never found in the mid-gut of the flea, the infection always being confined to the hind-gut and rectum, while occasionally the Malpighian tubules are involved. Usually parasitization commences abruptly behind the pyloric opening and extends downwards according to the intensity of the infection. The forms found in the Malpighian tubules are long, active leptomonads, similar to those first found in the mid-gut of larvae: they are about  $23\mu$  long and exhibit twists. They are usually found near the distal extremities of the tubules. The rounded forms described by PATTOX and RAO (1921) as occurring in the Malpighian tubules of the human flea *Pulex irritans* have not been found in the present species. In order to explain the presence of immotile forms in the tubules, these authors suggest that they have probably been carried there by the active flagellates. WRETTON (1926) regards the theory

as improbable, but an instance has been noted recently (GIBBS, 1942), in which leptomonads and crithidia carried about with them a number of leishmania-like forms adherent to the flagella and never seen to become detached. The forms found in the gut and rectum are (a) stumpy leptomonads, (b) rounded leishmania-like forms, and (c) smaller non-flagellate bodies. Leptomonads about  $55\mu$  long with short flagella constitute the majority of the parasites in the gut and rectum (Fig 4). They are never found free within the lumen, anchorage always being afforded by the flagellum, but there is frequently a pendulum like swaying of the body. They are sluggish



when liberated into saline, there is movement of the flagella but they do not travel and are never found with the twists which are characteristic of leptomonads which infect the mid gut of larvae.

The larger rounded forms measure about  $5\mu$  across and are often found with short flagella, indicating development to or from leptomonad form. The nucleus is usually eccentrically situated and the kinetoplast is large, the rhizoplast is very distinct (Fig 5). They are frequently found in a state of division and rosette formation is common.

The small, ovoid leishmania-like forms are never found dividing. These are the resistant, infective bodies which are present in the dejecta of infected fleas.

WENION (1926) states that when there is little nourishment in the gut practically all the flagellates are in the attached condition, but after a long

meal of blood, many active forms can be seen within the gut contents. During the present investigation free forms have never been observed within the gut, even after a meal. WEXTON also states that towards the posterior end of the intestine the attached flagellates, and also those free within the cavity become smaller till finally little ovoid leishmanial forms are produced. It has not been possible to confirm this statement as no consistent differentiation has been noted between the parasites situated just behind the pyloric opening and those found at any other part of the hind-gut or within the rectum. It will subsequently be shown, also that the smaller leishmanial forms (the resistant bodies) which are described by SMOYER (1923) as occurring only in the rectum, are also to be found at the upper end of the hind-gut.

#### DEVELOPMENT OF RESISTANT BODIES IN SALINE.

WEXTON (1914) noted that in some form the parasite is resistant to desiccation, as he obtained growth in culture from material which had been dried for 24 hours, and concluded that the resistant forms are the small leishmania-like bodies. It has been found that the development of the resistant body takes place in normal saline and it is thus possible to follow directly the subsequent flagellation and change of form of the organism. The period of viability of the bodies while contained in dried faecal blood is considerable an interval of 2 months does not in any way appear to affect development.

Resistant bodies of *Leptomonas ctenocephali* can be obtained for study in two ways. Firstly they can be found in the faeces of infected fleas which has been dissolved in saline and, secondly they can be obtained directly from the hind-gut and rectum by dissection. In the first case the faecal blood can be dissolved under a cover-glass and observation commenced immediately. If the saline is considerably hypotonic the bodies disintegrate owing to osmotic pressure differences while if the blood is dissolved in water disintegration is immediate and complete. In the second case the gut and rectum can be removed split lengthwise and laid on a slide to dry. Under these conditions the bodies survive for about 5 hours, even when gentle warmth has been applied for a short time to ensure thorough drying. When examined later in saline during the period of viability they can be easily distinguished from all other types by their smooth appearance, which contrasts sharply with the vacuolated condition of the dead flagellates and larger leishmania-like forms. It is found that the resistant bodies obtained from the flea by dissection survive for a period of 3 days when they are dried in a solution of saline and abattoir blood.

The development of the resistant bodies in saline takes the same form whether passed out naturally with the faeces, or obtained from the gut or rectum of the insect by dissection. At first they lie at the bottom of the saline

and it is not until the flagellum is fairly active that they commence to rise. In a period varying from 10 to 30 minutes the first indications of the growth of a flagellum can be detected. Fig 6 shows a stained specimen in which the flagellum has commenced to protrude from the body. At first the filament is non-motile, but when it attains a length equal to one-half of the body-length, it begins a jerky, side to side movement which increases in vigour with growth. Simultaneously the body elongates and the parasite assumes leptomonad form. Fig 7 shows a stained leptomonad after 30 to 60 minutes in saline. In 60 to 120 minutes the body becomes twisted (Fig 8), and soon afterwards it attains a length of approximately  $13\ \mu$  with a long flagellum. At this stage the parasite, although smaller, is similar to the first forms which infect the mid-gut of the larva, and is highly motile. Dividing forms can be found after some hours. Development then ceases, although the flagellates may continue to be active for 24 hours.

The resistant bodies can be found at the upper end of the hind-gut as well as in the posterior portion and in the rectum. This has been established by the following often repeated experiment. The alimentary tract was removed from an infected flea and the hind-gut cut as closely as possible to the pyloric opening. The remaining length of gut and the rectum was discarded, while the short length taken from just behind the mid-gut was placed on a clean slide and dried. When examined later in saline many of the infective bodies could be found, and these afterwards developed into motile forms. It has been found that when artificially removed parasites are dried for a period not exceeding about 15 minutes, a few flagellate forms revive within a minute or two in saline, while the resistant bodies flagellate later.

#### SURVIVAL UNDER ADVERSE CONDITIONS OF TEMPERATURE, ETC

Experiments were undertaken to study the effect of various temperatures on the resistant bodies contained in the faeces of fleas. No effect on development is to be noted after refrigerating the material at a temperature of  $0^{\circ}\text{C}$  for 24 hours. After a similar period at  $45^{\circ}\text{C}$  there is a lengthening of the time taken to become motile (1 hour), while certain individuals fail to become as active as usual. Larvae which feed on the blood become infected when the blood is heated to  $65^{\circ}\text{C}$  for 24 hours only about  $1\frac{1}{2}$  per cent of the bodies develop in saline, while the time taken to become motile is increased to 5 hours. They are very feebly active and are incapable of rising from the bottom of the liquid. Development does not progress beyond the very stumpy leptomonad form. Larvae fed upon this material do not become infected. Twenty-four hours at a temperature of  $70^{\circ}\text{C}$  was found to be lethal. Granules of faecal blood which have been immersed in absolute alcohol for 30 minutes will yield active flagellates when dissolved in saline.

placed immediately on the stain covered slide and care taken to see that the film of blood spreads uniformly between the slide and the coverlip. If necessary the coverlip is gently pressed with finger. This little procedure is very helpful for the uniform spreading of the blood and can be learnt in only a few minutes. It however should be done only once as it is important not to disturb the coverlip any more, otherwise a precipitate might form. The slide is allowed to stay at room temperature for 10 minutes and examined with  $1/12$  in oil immersion lens. The chromatin of the parasite stains chocolate red the cytoplasm deep blue and the corpuscle light green. The contrast is so marked that the parasite if any can be immediately picked out at a glance and no elaborate searching of individual corpuscles is necessary as in Romanowsky-stained thin smears. Microscopic fields not having the parasite can simply be skipped over at a glance. There is very little or no distortion of the parasites or the red cells, and hence the former can be studied practically in their natural state. This is in great contrast to the usual thick smear preparations where the cells are subjected to rather violent methods of dehaemoglobinization and staining. It practically combines all the advantages of a thick smear without the latter's drawbacks. There is no necessity for washing, drying and other procedures, and hence it can be claimed that the method is the most simple one. If we take into consideration the fact that all the above procedures have been eliminated—the actual interval between the taking of blood and its microscopical examination as well as the finding of malarial parasites, is at least as short as the quickest method now in vogue. Owing to the simplicity of the method it is most suitable where mass-examination of blood is necessary as in outdoor dispensaries, relief work centres, etc. Prepared slides can be carried to the field in slide boxes and all that the workers have got to do is to touch a drop of blood on the coverlip, place it on a slide and examine after 10 minutes. There is no necessity for drying, dehaemoglobinizing, pouring of stains, washing, etc., or of carrying their necessary paraphernalia to the field. Another great advantage is that the reticulocytes are stained at the same time. These can be counted easily thereby giving valuable information about the state of regeneration of the erythrocytes. No application of vaseline is necessary at the margin of the coverlip as the blood dries up in this region and by itself forms a sort of sealing material. The only drawback of this method is that the slides cannot be preserved, and it is better to examine them within 24 hours.

For the sake of brevity neither the history nor discussion of details of the various methods of staining blood smears are given here. For this purpose reference may be made to papers by the following authors—ESTLIN (1879), ROMANOWSKY (1891), UCHIDA (1891), LEISHMAN (1901), MCNEAL (1906, 1925), HOLMES and FRENCH (1926), COOK (1930), FIELD (1941), WILCOX (1942), SEVEN and BRATTAGLIA (1944).

## SUMMARY

1 By means of supravital staining the malarial parasites can be sharply stained and easily found out. The stain consists of Leishman's stain 2 parts, brilliant cresyl blue 1 part (1 per cent alcoholic solution)

2 The method eliminates fixing, dehaemoglobinisation, drying, changing of stains and washing

3 Reticulocytes may be counted at the same time

4 It is a better contrast stain than the usual Romanowsky methods

5 There is no distortion of the corpuscle or parasites such as is found in thick smears

6 It is a very suitable method for mass examination of blood in outdoor dispensaries, relief centres, etc

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## CORRESPONDENCE.

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### CASE OF SEVERE REACTION TO ANTRYPOL

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*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

In November, 1943, I was hunting elephant along the banks of the River Volta on the French side of the river at the level of the town of Bole. This territory has been almost completely depopulated through trypanosomiasis, and my headquarters were at a small fishing village which had been established on the bank of the river a year and a half previously. At this village, the three children of the chief all had masses of enlarged glands in the neck and were eventually treated for trypanosomiasis by Dr STACEY-MORRIS at Lawra.

One day, accompanied by two African soldiers and two guides, I came across the bedding down site of a herd of bush cow, where we were almost immediately attacked by swarms of very hungry tsetse flies. They assailed us from all directions, and in a matter of 5 minutes we were all bleeding from scores of puncture holes. We ran for nearly 2 miles in single file, with everyone beating the back and legs of the person in front with twigs to keep away the flies, but, in spite of this, I was myself bitten perhaps a hundred times, in the neck, through my shirt, trousers, stockings, and even on the palms of the hands.

One fly worked itself through a hole in my stocking and proceeded to draw blood until I squashed it against my leg. We finally reached the river and took refuge in the water.

Two days later the place where I had squashed this particular tsetse fly began to swell, and by the time I had reached my main base at Kumasi, 9 days later the place where the tsetse fly had bitten me was a papule surrounded by a raised white area some 3 to 4 inches in diameter and beyond that the leg was reddened and the muscles stiff.

At Kumasi a member of the trypanosomiasis team scraped the papule and demonstrated a living trypanosome in the exudate. A film was made and stained with Leishman, and again, after much searching, the trypanosome could be seen. A blood film was negative.

Treatment was given at the Military Hospital Sunyani Road, Kumasi, by the medical specialist, and consisted of a small dose of antypol to test my sensitivity to same, and then a second dose intravenously. My two African soldiers were given the same, except that their second dose was given intramuscularly. The antypol in the second dose came from a bottle which had been opened 3 days previously and it was later suggested by Brigadier FINDLAY that the antypol had decomposed or had been oxidized to some extent. Whatever the explanation, within 15 minutes of the injection I was conscious of tingling all over the body and proceeded to have a violent rigor. There was some vomiting and many scotomata in both visual fields. I finally became unconscious, and remained in this state for 48 hours.

The two Africans had reactions not so severe as my own, but both were confined to bed for 2 weeks with legs very painful and stiff around the sites of their intramuscular injections.

On recovering from this mishap, I suffered from considerable lassitude and weakness for several months but was delighted to find that the swelling on my leg had disappeared during my period of unconsciousness. There have never been any enlarged glands in my neck. My blood, which has been examined at 6-monthly intervals, has never been found to contain any trypanosomes. At the site of the papule there remains a small round, hard, fibrous nodule about the size of a pea.

I am, etc.,

T. A. COCKBURN.

Zoological Society of London.

## POROCEPHALOSIS

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

There can be no doubt that linguatulid infestation in man is occasionally capable of producing quite serious symptoms

Dr STOCK's paper (1946) in these *TRANSACTIONS*, on a case of collapse of lung associated with the presence of a calcified nymph of *Armullifer armulatus* in the vicinity of a bronchus recalls to mind a case which came to my notice in Enugu, south-eastern Nigeria, about 4 years ago. The patient was an Ibo woman aged about 40 who was admitted as a surgical emergency suffering from acute intestinal obstruction. The cause of the obstruction was found at operation to be a narrow fibrous band placed transversely across a coil of lower ileum, which it kinked as well as strangulated. It extended from the root of the mesentery, across the intestine, and was attached to a calcified nodule the size of a large pea on the side wall of the pelvis. There was well marked, localized degenerative fibrous tissue reaction. The ileum itself was apparently otherwise normal. Division of the band immediately relieved the obstruction. The patient made an uninterrupted recovery. The "nodule" was later identified by the Medical Research Institute, Yaba, as a calcified nymph of *A. armillatus*. Cellular reaction following the death and calcification of this single parasite had no doubt been responsible for the formation of the "band" and consequent obstruction.

*Armullifer* infestation of man is undoubtedly more common than the sixteen cases recorded up to now in the literature would appear to suggest. During 19 years' practice in Southern Nigeria, I have seen five cases. Cases 1 and 2 (unpublished) were seen in an inguinal hernial sac at operation. Cases 3 and 4 were, as in the majority of the reported cases, diagnosed only at autopsy (MANUWA, 1928 and 1935). Case 5 is the present one. All were natives of tribes well known for their habits of eating snakes or of using live or dead snakes in the practice of various forms of fetish ("jugu").

From the available evidence and from personal observations, the position with regard to the pathogenicity of these parasites may, I believe, be briefly summarized as follows —

(1) Generally speaking, infestation is symptomless, if the nymphs are few and remain quiescent and encysted. Even so, heavy parasitization of a vital organ, *e.g.*, the liver, may produce serious symptoms. In Dr LINCOLN BELL's case, reported by CANNON (1942), an overwhelming infestation of the walls of the colon produced intestinal obstruction and death.

(2) Considerable acute pathological lesions leading to death of the host may be produced if the nymphs rupture their cyst-envelopes, escape, and

begin to migrate. In my Case 4 in which the symptoms suggested acute poisoning, nymphs were demonstrated in the radicles of the hepatic vein and were evidently in the actual process of migrating from the liver.

(3) Calcareous and degenerative changes occurring around deceased nymphs may depending on their anatomical position, produce obstructive or other symptoms. Dr STOCK's case and my Case 5 are examples.

(4) There is no evidence that the parasites produce toxic symptoms.

Dr STOCK's case is the second one in which these parasites have been radiologically demonstrated. In LOW and CORDINER's (1935) case, calcified nymphal cysts were radiologically identified in the liver mesentery and lung. Diagnosis in the living subject is otherwise unusual.

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I am etc.

S. L. A. MANUWA.

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#### CEREBRAL LESIONS IN DOGS FOLLOWING INJECTIONS OF 4-4 DIAMIDINO-STILBENE.

To the Editor *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

Sir,

I have read the paper on the above subject by OASTLER and FINER published in your journal (*Trans R Soc trop Med Hyg* 39: 533) with considerable interest. The writers have been able to produce certain symptoms and pathological changes in the brain of the dog by the injections of what they have called 'fresh' solutions of stilbamidine. They have suggested that these experimental lesions may throw some light on the pathogenesis of certain neuropathic signs and symptoms described after the use of the drug in man. But the neurological changes they have described are entirely unlike what may be expected in diamidino-stilbene neuropathy—the neurological sequel to stilbamidine therapy first described by NAPIER and SEV CEPTA in 1942.

In the course of my investigations on the cumulative toxic action of stilbamidine since 1942, I have administered courses of injections of this drug to the rhesus monkey and the rabbit in 2 to 5 mg per kg bodyweight as single doses and one complete course consisting of 15 to 20 injections given on 6 days a week. In no case, either in the monkey or in the rabbit, was any symptom produced bearing the slightest resemblance to those described by OASTLER and FIDLER in their experimental dog. The only symptoms that were noticed in the monkey were flushing of the face, at times deep breathing, and occasionally closing of the eyes and lowering of the head immediately after the injection was given. During, and for 3 to 4 months after, the course of injections the monkeys remained in perfect health. When these monkeys were destroyed either by air embolism or intravenous injection of a cardiac glucoside no obvious changes were noticed either in the brain substance or the meninges anatomically. On histological examination after staining with haematoxylin and eosin, no changes were to be seen in the brain or in blood vessels including those of the entire region of the internal capsule, thalamus and the caudate nucleus.

In the rabbits that were given rather larger doses of previously heated solutions of the drug, there was a rapid loss of weight and death was caused by great damage to the liver and the kidney mainly and there was seen wide spread degenerative changes and haemorrhages in different internal organs. The nervous tissues, however, did not show any gross abnormality and on histological examination after staining with haematoxylin and eosin no vascular abnormality or haemorrhages or perivascular accumulation of inflammatory exudate was seen.

It should also be mentioned that until now over 200 cases of Indian kala azar have so far been treated with stilbamidine at the Calcutta School of Tropical Medicine using 1 mg per pound body weight as the maximum single dose and a single course of injections consisting of ten to fifteen injections given mostly on consecutive days. Some of the patients had to be given two to three courses of injections at intervals of 2 to 3 weeks. Symptoms as described by OASTLER and FIDLER or suggestive of any lesion of the nervous system were entirely absent during or immediately after the course of injections or for that matter during the stay in the hospital after the completion of the treatment. Only in a fair proportion of the cases was there development of diamidino-stilbene neuropathy 2 to 4 months after their discharge from the hospital.

It appears that OASTLER and FIDLER had used solutions of stilbamidine that had been autoclaved at 5 lb pressure for 20 minutes. It is well known that aqueous solutions of stilbamidine are liable to chemical changes which lead to great increase in toxicity. It is quite possible that such changes may be induced by autoclaving under pressure a solution of stilbamidine for such a prolonged period. It is hardly correct to regard an autoclaved solution as

fresh. As a matter of fact autoclaving was not necessary: solution of the contents of an ampoule of stilbamidine in sterile distilled water gives a sterile solution suitable for intravenous injection.

The lesions described by OASTLER and FIDLER in some of the dogs closely resemble that seen in acute infective inflammation of the brain and meninges, so great were the vascular changes and the infiltration with polymorphonuclear leucocytes. It is, however, hard to realize how neuronie or myelin degeneration could be fully studied without using Weigert-Pal or similar stains or special staining for Nissle's granules, the authors having used only routine haematoxylin-eosin stain.

In conclusion it may be pointed out that the pathological changes produced by OASTLER and FIDLER should be regarded as caused by the injections of autoclaved solutions of stilbamidine in the dog taking for granted that there has been no accidental superimposition of some infection. I must confess that the observations of these writers do not in any way help in understanding the pathogenesis of diamidino-stilbene neuropathy. Vascular damage and haemorrhages undoubtedly occur after poisoning with heated solutions of stilbamidine in the rabbit, but it is very doubtful whether this type of lesion or that described by OASTLER and FIDLER can selectively affect the blood supply of a part of the fifth nerve nucleus. So far the evidence from my experiments with the rhesus monkey is against the occurrence of any such lesions.

I am, etc.,

P. C. SEN GUPTA,

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# CORRIGENDUM

VOL. 40, No. 3, PP. 275-294

Paper by E. GRAMET (1946) Control of plague by means of live avirulent plague vaccine in Southern Africa on pages 277 et seq  
for *Salmonella pestis* and *S. pestis*  
read *Pasteurella pestis* and *P. pestis* respectively

Printed in Great Britain by H. R. GOSWAMI, LTD. Calcutta.

*The previous number of these Transactions, Vol 40, No 4  
was published on March 5th 1947 ]*

# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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VOL 40 No 5 MAY, 1947

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ORDINARY MEETING  
of the Society held at  
Manson House, 26, Portland Place, London, W ,  
on  
Thursday, 16th January, 1947, at 8 p m

THE PRESIDENT,  
C M WENYON, CMG, CBE, MB, BS, BSC, FRCS,  
in the Chair

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## PAPER.

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### RECENT KNOWLEDGE ABOUT MALARIA VECTORS IN WEST AFRICA AND THEIR CONTROL

BY  
R C MUIRHEAD THOMSON, D SC

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## INTRODUCTION.

In this paper I should like to give you a brief outline of some of the work that has been going on in West Africa for the last few years in connection with malaria transmission and control. The rapid advances that have been made in the last 5 or 6 years are partly due to the important, and at times vital, part West Africa played in the supply line to the Middle East and North Africa during the war and partly due to the increasing funds made available by the Colonial Development and Welfare Act of 1940 for anti-malaria work and trained personnel.

In this work many different people have played a part, both military and civil British and American, and it may all be regarded as a combined operation in the widest sense. Much of this work has been published, but a great deal has not yet appeared in print, and it is probable that much of that will remain tied up in the original typescript or cyclostyled report form and never become available for reference. This multiplicity of reports and opinions makes it rather difficult for the onlooker to get a clear idea of what has been happening and is happening just now and although what I offer today is just one more report, one more opinion, I hope that it may help to clarify the mosquito aspect of the problem at least.

Without describing in detail the geography of West Africa, there is one feature which is apt to be overlooked and one which has a bearing on anti-malaria policy. While it is often convenient to think of British West Africa as a single homogeneous unit in actual fact the four colonies, Gambia, Sierra Leone, Gold Coast, and Nigeria, each with its separate Colonial Government, still remain very distinct, widely separated by non-British territory and peopled by races whose interests have little in common. Communication between the four colonies is almost entirely by air and sea, and the distances to be covered are still large. Added to this is the fact that while the topography of the four colonies has a great deal in common, the differences, even in the coastal belt, are still wide enough to make generalization dangerous. The rocky laterite

Colony of Sierra Leone with its remarkable rainfall obviously presents features very different from Gold Coast with its bare undulating coast of reddish loam and from Lagos with its porous sandy soil vast lagoons and coastal swamps.

As a result of all these factors malaria control policy in the last few years has pursued a more or less independent course in each colony and while a great deal has probably been lost by this lack of interchange of ideas and methods, each colony does present its own peculiar problems which can only be tackled successfully in the light of local knowledge.

Since 1942 I have spent nearly 2 years in Sierra Leone, mostly in Free town estuary and 18 months in Lagos. I have also paid a short visit to Accra, Gold Coast, and Bathurst, Gambia. I can only speak with any degree of confidence about coastal districts but, however important the hinterland may be

as far as anti-malaria work is concerned it is the coast, on which the capitals of the four colonies are situated, that will be our main concern for some time to come

### IDENTITY OF *Anopheles gambiae* AND *A. melas*

It has been known for many years that the main vector of malaria in West Africa is *A. gambiae*, with *A. funestus* of secondary importance, and that these two mosquitoes together are mainly responsible for malaria transmission throughout tropical Africa

In West Africa it has also been known for some time that in coastal districts a dark form of *A. gambiae* also occurred. In its most distinct form this dark or melanic variety was called *A. gambiae* var *melas* and differed from typical *gambiae* in having an additional dark band on the palps. Up till about 1940 this was regarded as little more than a "melanic coastal variety," associated with salt water breeding places. Dr BARBER (1931) in his Nigerian survey had reported finding infections among these dark forms, but nothing more was known about its exact identity or its role in malaria transmission (EVANS, 1931, 1938)

Early in the war Professor BLACKLOCK and Dr CARMICHAEL WILSON (1941), in their investigation of malaria among merchant seamen found odd *melas* in Freetown itself, and large numbers of them on the Bullom shore, across the estuary from Freetown. In these Bullom villages they found a high rate of infection among the "var *melas*," and considered it must play an important part in malaria transmission in the estuary.

About this time, too, house catches in various parts of Freetown estuary were started by the Field Hygiene Section, and later continued by the Malaria Field Laboratory, in connection with airfield and seaplane base construction, and other military installations. They showed regular catches of this "var *melas*" from most parts of the estuary except Freetown proper, and its neighbouring village or suburb of Kissy.

The next step was when Major RIBBANDS (1944) found that of mosquitoes bred out from eggs laid by distinct "banded" *melas*, only about one-third of the females were banded, the remaining two-thirds being identical to typical *gambiae*. From this it appeared that "var *melas*" must include not only those dark forms, with distinct four banded palps, but also some mosquitoes which had hitherto been regarded as typical *gambiae*. RIBBANDS also, following up BARBER's original observations, found that larvae of *gambiae* and "var *melas*" showed distinct differences in their reaction to sudden changes in salinity, and a simple test was devised in which larvae were exposed to changes of increasing salinity in such a way that most of the pure *gambiae* larvae died, and most of the *melas* survived (RIBBANDS, 1944).

This physiological difference was followed by a morphological one, the pecten of *melas* and *gambiae* larvae showing consistent differences in relation of the teeth (RIBBANDS, 1944).

The situation at this stage was that when we found adults with the extra dark band on the palps we knew we were dealing almost certainly with *melas*, but where there was no extra dark band we did not know whether we were dealing with unbanded *melas* or with typical *gambiae*. Then in 1942 we discovered that the eggs of the two forms were quite distinct and that the well-marked differences were consistent (Muirhead Thomson 1945). Here at last we had a method of knowing exactly what mosquitoes we were dealing with. When we caught mosquitoes in villages the banded ones were practically all *melas* while the unbanded ones which might be *melas* or *gambiae* were put aside to lay eggs on which the identity of the mosquito was established. The egg character remains the only accurate means we have of identifying individual wild-caught females on the coast. Robertson (1943) has found that the range of variation in certain tibial markings is different in the two species, but we still lack any morphological feature which can be used to distinguish all adult *gambiae* from all adult *melas*.

We now felt that with so many differences to go on we could regard *A. gambiae* and *A. melas* as distinct species (Riechards, 1944; Muirhead Thomson 1945).

In the last 18 months in Lagos we have been able to carry the business a stage further. In captivity *gambiae* and *melas* which have been bred out in the laboratory show no inclination to mate. Until this happened we could not offer proof that the two species breed perfectly true to type. In Sierra Leone we tried small cages full of *gambiae* or *melas*, large cages, inside and outside at dusk and fed the females regularly on blood. But there was still no mating and fertilization, the ovaries did not develop, and no eggs were laid. The same methods were tried out in Lagos using large numbers of laboratory-bred mosquitoes, but still nothing happened, till finally one night the cage was kept illuminated by a table lamp with an orange coloured shade for several hours at night. Under those conditions both *gambiae* and *melas* mated readily and laid eggs after a blood meal. In this way we could finally show that adults bred from *melas* eggs in turn laid nothing but *melas* eggs and *gambiae* also remained true to type. One of these laboratory-bred and mated *gambiae* females lived 86 days in a small 6 by 6 by 6 inch cage. In that time it took thirty blood meals and laid twenty-eight batches of eggs, all of them distinct *gambiae* type.

In order to bring our observations into line with those of the European *A. maculipennis* complex, we persuaded *melas* males to fertilize *gambiae* females, and *gambiae* males to fertilize *melas* females, illuminating the cages as described above. The eggs laid in a cull of these cross matings showed no transitional forms but were determined by the identity of the female. These eggs produced healthy larvae which gave rise to a hybrid generation of healthy adults. Attempts to get these hybrid to mate with each other or with pure *gambiae* and *melas* were unsuccessful, and examination showed that while the

females were apparently normal sexually, the males were in most cases sterile, with underdeveloped or atrophied testes.

The sterility of hybrids is a severe test of specific status, and considered in conjunction with all the morphological and physiological differences just described forms a pretty firm basis for regarding *gambiae* and *melas* as two entirely distinct species.

#### MALARIA TRANSMISSION BY *melas* AND *gambiae*

However satisfactory it may be to have established the exact identity of *melas* and *gambiae* in West Africa at this stage one naturally asks whether it is anything more than a nice piece of pure systematics, and whether it has any bearing on the problem of malaria control. On this point I can assure you that on many parts of the coast the efficiency of control is and will be to an increasing extent, determined by the full appreciation of the relative abundance and relative infectivity of the two species in the area.

In Freetown estuary we are fortunate in that about one third of the *melas* have distinctly banded palps, and we can form a rough idea of its incidence in different places by catching mosquitoes in houses and multiplying the number of four banded females by three to give an estimate of the total *melas* in the catch. This is a rough and ready method which can only reasonably be applied to large samples. In small catches it may lead us astray. But by this means we could tell, for example, that while *melas* was abundant in most of Freetown estuary, Freetown itself and the neighbouring village of Kissi were almost pure *gambiae* areas.

In and around Lagos, on the other hand, only about 5 per cent of the *melas* have this distinct extra dark band, the remaining 95 per cent being indistinguishable from typical *gambiae*. In this case we must rely entirely on egg characters before we can have any idea at all about the relative abundance of *melas* and *gambiae* in houses.

This was one of the first things to be tackled in Lagos. From January, 1945, onwards several catching stations were selected in and around Lagos, and at intervals mosquitoes were collected by hand-catching from these village and town houses. Of the total collected a sample of anything up to fifty females were put aside, one mosquito to a cage and they were identified by the eggs they laid during the following 2 or 3 days. As *melas* and *gambiae* lay equally well in the laboratory we could work out fairly accurately the number of *melas* and *gambiae* in the catch. From these records we now have for the first time some idea of the incidence of the two species in several different localities round Lagos, and the relative abundance month by month right through the season, based on about 15,000 adults collected, and about 7,000 egg batches identified.

This is rather a laborious method but there is no other way of acquiring such figures at the moment, and the results amply repay time and labour.

For example, in one village surrounded by hundreds of acres of mangrove and brackish swamp, we find that at certain times of the year most of the mosquitoes in the houses are not *melas* as we might expect, but *gambiae* sometimes originating from a single freshwater borrow pit beside the village.

Lagos town itself is situated on an island on which there are no longer brackish swamps, but just three-quarters of a mile across the harbour there is a great production of *melas* for many months of the year from the extensive tidal swamps. Yet 96 per cent. of the house catch in Lagos is *gambiae* which originate almost entirely from local rain-filled breeding places. Similarly just about a mile or two inland from the *melas* infested coastal swamps one finds villages in which the population is entirely *gambiae*.

In Freetown estuary too, egg identification has revealed sudden changes in the mosquito population which might otherwise have escaped our notice. Wellington village, where much of our work on breeding habits of *melas* was carried out, is dominated by *melas* for the greater part of the year. In the early rains in June there is a great peak of mosquito production corresponding to intense breeding of *melas* in the adjacent *Avicennia* orchard. Egg identification however showed that at this peak period which only lasts a few weeks, about half the mosquitoes in the houses are *gambiae* originating from numerous rain-filled pools in among the village houses.

As results of control measures in all these areas become more and more evident, it will be increasingly important to know the identity of mosquitoes still occurring in small numbers in houses, so that time and money are not wasted in controlling the wrong breeding places.

There is a great need for more information of this kind on the coast, and it can only be done by routine egg identification of mosquitoes from representative catching stations. For example, we have no idea of the relative abundance of *melas* and *gambiae* in the other two colonial capitals, Accra and Bathurst. It is possible that they may both be affected by extensive tidal swamps nearby or it is possible that local breeding of *gambiae* in freshwater pools is the main concern. Until such information is forthcoming we have to rely on conjecture, which is definitely not conducive to sound malaria control.

We have already referred to the records of BARBER and OLDWICK (1931) who found infections among melanotic coastal form of *gambiae* presumably *melas*, in the Lagos area. In Sierra Leone, too, BLACKLOCK and WILSON (1941) found four sporozoite infections in seventy-four "var *melas*" on the Bullom shore and they considered that the high infection rate both of malaria parasites and microfilariae was of considerable importance for West Africa.

On the other hand there were reports from East Africa that MACKAY (1938) found no infection in "var *melas*" from Dar-es-Salaam. In this connection I might point out that nothing was known at that time about the identity of *melas* in East Africa, and as far as I know the situation is equally vague today.

Once the identity of *melas* as distinct from *gambiae* had been established

it was felt that a new series of dissections of material identified both by palpal banding and egg characters was desirable. As a result we have the records of 1,000 dissections carried out by No. 5 Malaria Field Laboratory under Dr TREDRE, on material from Freetown estuary. These *melas* showed a sporozoite rate of 4.2 per cent for the whole season. This is much lower than the usual figures for *gambiae* from this area (GORDON *et al.* 1932), but the figures were not strictly comparable as the records were from different localities.

This question was taken up in more detail recently in the Lagos area where all identifications were based on egg characters. There we have records from many localities at all months of the year, but what is more important, we were fortunate enough to find the type of village which we had looked for in vain near Freetown, namely one in which both *melas* and *gambiae* occurred together in numbers in the village houses throughout most of the year. We could now test the infectivity of *gambiae* and *melas* when they were exposed to chances of infection as equal as it is possible to find in nature. The results showed that under those equal conditions *gambiae* with a mean sporozoite rate of 11.1 per cent is nearly three times as efficient a vector as *melas*, with a mean rate of 4.5 per cent. These consistent differences were also shown in all other catching stations, the combined figures for sporozoite rate being 10.0 per cent for *gambiae*, and 3.5 per cent for *melas*.

Furthermore, at certain times of the year the sporozoite rates of small samples of *gambiae* reached heights never approached by *melas*. The highest figure for any month was a sporozoite rate of 8 per cent for *melas*, compared with the remarkable figure of 29 per cent for *gambiae*. This latter figure is just a little short of the "epidemic" infection found in *gambiae* after its introduction to Brazil and also agrees closely with BARBER's findings in those localities in which he, unknowingly, was working with pure *gambiae*. Although *gambiae* remains unique in this respect, it does not alter the fact that *melas* is a pretty efficient vector, too, and that the two of them form a very formidable partnership on the West African coast at least.

As has been emphasized earlier in this section, the recognition of the parts played by *melas* and *gambiae* in malaria transmission in any locality in West Africa is an essential basis for sound control policy. The relative importance of the two varies greatly from place to place, even between one end of a town and the other, and so far it is only in the Lagos area and in Freetown estuary that we have the necessary information. As we shall now see the breeding places and control of the two species are so strikingly different that effective control against one species alone may leave the problem of the other practically untouched.

#### BREEDING PLACES OF *Anopheles melas*

I shall deal first with *melas*, as it is with this anopheline that most of our knowledge has only recently been acquired.

The capacity for *var melas* to breed in brackish water in coastal swamps has been known for many years. BARBER and OLINICK in 1930 found it breeding among *paspalum*, which is a coarse sea grass covering great stretches of tidal swamp near Lagos.

In Freetown the first brackish water breeding place was also found in a marshy patch of *paspalum* and other grass in an area outside the mangroves and only flooded at spring tides (RICHARDS 1944). But brackish swamps of this kind are scarce in Freetown estuary where most of the great area between low tide mark and high spring tide mark is covered with dense mangrove. And yet in those pure mangrove areas with few open spaces of *paspalum* swamp we were finding high catches of *melas* in the village houses. In the one known breeding place described above which lies just west of Freetown, larvae were never found extending from the sea grass into the adjacent mangrove. As this was regarded as a typical *melas* breeding place the search for similar types of swamp went on for months with completely negative results.

Finally we found a few larvae in a part of the mangrove belt where the mangrove appeared to grow in rather a different way from usual, spaced out on very flat and fairly firm ground, rather like little apple trees. To cut a long story short these characteristic orchards as we called them belonged to those of the black mangrove, *Avicennia* while the great bulk of the mangrove belt is composed of the more familiar red mangrove, *Rhizophora*, with its stilt roots, aerial shoots, and dense tangled growth. Dense breeding of *melas* was soon found to be confined to those *Avicennia* orchards, whereas the great stretches of pure *Rhizophora* and their fringes of *paspalum*, were harmless. (MUTTERHEAD THOMSON 1945).

These *Avicennia* orchards are only flooded by the spring tides, and vary greatly in size anything up to 50 acres. At certain times enormous numbers of *melas* larvae may be found scattered right through the orchard.

Now it was clear that the presence of one of these great orchards near the anchorage or seaplane base might nullify the anti-malaria work going on ashore. Searching for the breeding grounds by exploring on foot in the mangroves is a tedious job especially with the prospect of about 20 miles of coastline covered with mangrove anything up to 5 miles deep. When viewed from high ground the great mangrove belt appears a uniform green in the rainy season, but in the dry season the *Avicennia* orchards can be picked out by the light green colour of their foliage, compared with the various darker shades of *Rhizophora*. Aerial reconnaissance was then tried, and new orchards were seen in this way although the method had its drawbacks in that it was very difficult to get one bearing even looking out of a comparatively slow moving Swordfish plane.

Finally the method of aerial photography was tried. The R.A.F. had already a fairly complete mosaic of the estuary made up of vertical aerial photographs but in this mosaic it was very difficult to distinguish the differences in foliage texture and shade. But when the original prints were enlarged

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twice everything stood out clearly, and we could pick out and define the total area of *Avicennia* orchards right round the estuary. In this way we saw that the *phora*, and later work showed that only about six of these were of first importance as breeding places.

Now these orchards are only flooded by the spring tides covering them twice a day for 4 or 5 days each fortnight, or each month in the dry weather, and as we might expect it is the periodic flooding with salt water that produces the breeding places. But what was entirely unexpected was that the great crop of *melas* breeding starts almost as soon as the flat orchard is flooded by the first high spring tide, and that the larvae spend most of their 5 or 6 days larval life being flooded by the spring tides twice a day, often to a depth of 1 or 2 feet. When the spring tides subside, and the brackish pools are left undisturbed, the great crop of larvae are already full grown and pupating. If the same pools are visited a day or two later it may be almost impossible to find larvae.

Conditions in all the *Avicennia* orchards in Freetown estuary are almost identical in relation to flooding by spring tides and as a result for 2 or 3 days each fortnight, or each month in the dry season, there is an enormous peak output of *melas* from all the breeding grounds.

Fortunately, the tidal movements and heights in Freetown estuary are accurately known, and as a result we could bring our survey methods to an unusual pitch of refinement. Sitting in comparative comfort in the laboratory, with tide tables in one hand and the series of vertical aerial photographs in the other, it was possible to tell weeks in advance the exact day and hour on which an unexplored *Avicennia* orchard would have to be visited in order to determine whether or not it was an important breeding ground. If we went there on the wrong day we might find nothing, even in an important orchard, or we might find the place dry. Even on the very best day, if we visited the place an hour or two too early or too late we might find the place completely flooded by the high spring tide so that the larvae are widely distributed and difficult to detect, or we might find the tide so low that there was no way of reaching the breeding ground by canoe up the narrow creeks through the tangled belt of *Rhizophora*.

That clear cut picture unfortunately only applies, as far as we know at present, to Freetown estuary with its profuse growth of mangroves, and great deposits of silt where the two large rivers flow into the estuary.

In the low lying lagoon area of Lagos we find rather a different picture. There are few rivers bringing down silty water to the lagoon, the mangrove belt is much scantier and the inter-tidal zone is characterized by great stretches of *paspalum* sea grass. We find one or two *Avicennia* orchards like those of Freetown estuary, but we find *melas* breeding grounds in such a wide variety of places that *Avicennia* is no longer a guiding light. Furthermore,



tidal movements in the lagoon area are extremely variable, and their height actually unpredictable.

In Gold Coast, again, we find places like Accra where the coastal configuration is rather different, with *melas* breeding among the *paspalum* at the edges of small lagoons which may or may not be open to the sea at all times, and in which there may be no clear mangrove belt.

The true estuarine conditions found in Freetown estuary may possibly be duplicated in other parts of West Africa such as the Niger delta, but it is already clear that there is such a range of conditions that the findings in one part of the coast, no matter how clear cut and conclusive they are may actually be quite misleading if applied directly to some other part.

One other point about the breeding habits of *melas* is worth noting: that is, its almost complete restriction to the tidal belt. When the egg differences were discovered, the distribution of *A. melas* and *A. gambiae* breeding places in these coastal areas was studied by collecting eggs from a wide range of potential breeding places. In this way it was found that even in places where *melas* is enormously abundant, it makes use of freshwater pool only to a negligible extent. We have found a few eggs in rain-filled pools in Freetown itself and under very similar conditions in Lagos town. But for all practical purposes *melas* is rigidly confined to the area below high spring tide mark.

#### CONTROL OF *A. melas*

We have seen that in Freetown estuary dense breeding of *melas* is mainly confined to about half a dozen *Aticennis* orchards, whose total area is not more than 200 to 300 acres. This concentrated and clearly defined nature of the breeding grounds suggested that their control was a practical possibility.

These breeding ground tend to dry out at intervals during the neap tide periods, but drainage alone does not control breeding, because as we have seen, most of the larval development takes place while the ground is still being flooded twice a day by the spring tides. When the spring tide period is over and the drains start to act, the larvae are already full grown, or pupated and adults are produced before the ground dries up.

About the end of 1942 the idea of keeping the high spring tides out by bunding the breeding ground arose quite independently in Freetown and in Lagos, and despite the fundamental similarity of the problems the development of this method during the following 2 or 3 years pursued an independent course in the two colonies. The idea of reclaiming tidal swamp by building a bund or dyke to keep out the high spring tides and draining off the impounded water at low tide through automatic or hand-operated sluice-gates, is certainly not a new one. As far as West Africa goes I believe it was first used about 1939 or 1940 for reclaiming tidal swamps for rice cultivation in Sierra Leone. In that colony the malaria control models were based on this and the presence of an Irrigation and Drainage Department

with experience of empoldering seemed to offer a flying start to *melas* control. However, for various reasons, the progress in bunding has been disappointingly slow in Freetown estuary, and it is to Lagos that we must turn our attention to realize the enormous possibilities of this method of control.

In the Lagos area bunding has been carried out by Dr GILROY and his staff to an extent and with a thoroughness that forms a perfect working model for all malariologists who are likely to encounter this type of problem in any part of the world (GILROY and CHWATT, 1945).

In Lagos there is no question of clearly defined breeding grounds of limited area to be reclaimed. The aim here has been the comprehensive reclamation by bunding of the whole inter-tidal area with the exception of the outer fringe of mangrove lining the creeks and the lagoon. The very small tidal fluctuation in the Lagos lagoon, usually just a foot or two, has imposed less strain on the bunds, but at the same time made it more difficult to run off the impounded water from the flat swampland. As a result all sluice-gates are hand operated, the sills of the tide-gates are set 3 feet below mean sea level, and the internal drains are wide enough and deep enough to be of the nature of canals rather than drains. They always contain salt water, continuous with the lagoon water when the sluice is open, and clean edges. When well established they remain completely free of *melas* breeding. It is not just a matter of concentrating breeding into a small area, it is complete elimination of vast breeding grounds of *melas*. The technique in Lagos has been perfected to such a degree by Dr GILROY that I have seen apparently hopeless quagmires of brackish swamp and mangrove dried out in a matter of weeks. (In the last 4 years the total area of tidal swamp reclaimed in this way is about 5 square miles.)

To return to Freetown estuary there is an enormous tidal range, about 12 to 15 feet, compared with Lagos. This range should greatly facilitate running off the impounded water at low tide from the drains behind the bund, but at the same time the height of the spring tides, and their frequency, imposes a severe strain on the earth bunds, which evidently need to be constructed on a larger scale than in Lagos. Furthermore, the Freetown orchards, although of limited extent, are exposed to a much heavier rainfall than Lagos, to a heavy run-off of water from the hills of the dry land, and to heavy seepages from the dry land at swamp edge.

That is the sort of range of conditions one is likely to meet in the West Coast, and it indicates, I think, that while the principle of bunding has immense possibilities in eradicating *melas* from coastal towns and seaports, it may have to be modified considerably to suit local conditions. From what I have seen it is a method which seems to have great possibilities in the Gambia, near Bathurst. In the Gold Coast, as we have seen, the problem is mainly one of *melas* breeding in numerous small lagoons with ill-defined outlets on a surf pounded beach. Those who are familiar with the problem in that part do not advocate bunding, and the best method of dealing with such places has not yet been worked out. (BRUCE WILSON, 1946)

BREEDING PLACES AND CONTROL OF *A. gambiae*

The advances in knowledge about *gambiae* in West Africa in the last few years have naturally not been quite so striking as with *melas*. *Gambiae* is such has been familiar to generations of malarialogists and it remains probably the most dangerous anopheline in the world. In some ways it proves to be a remarkably versatile adversary while in other ways it appears to be a narrow specialist, with well defined likes and dislikes, unable to maintain itself unless things are just so.

As an example of its versatility we have some observations on the effect of artificial flushing of partly dried stream beds to eliminate *gambiae* breeding in pools. In the rocky colony of Sierra Leone there are some streams which provide ideal breeding places for *gambiae* in the dry weather when the water is reduced to shallow sunlit pools on the rocky bed. Artificial flushing of these stream beds, a control method which has proved very effective against such stream breeding mosquitoes as *A. minimus* and *A. fluviatilis* in India, seemed to be an ideal method for this particular problem. As a result dams and sluice gates were installed at the upper parts of some of these streams early in the war. One of the first things I was shown in Freetown was the flush in operation, and I was much impressed. A turbulent flood was released which swept this rocky stream bed from bank to bank. It appeared as if nothing could survive such a deluge and the flush seemed to satisfy all requirements. Unfortunately when the flood subsided after an hour or so, we still found large numbers of full grown *gambiae* larvae and pupae in the stream bed. After a time we found out that what was happening was this. The first flush washed the *gambiae* larvae out of their pools to the slack water at the edge where the larvae submerged and evidently lay quiet at the bottom. In fact we could show in the laboratory that *gambiae* larvae can survive complete immersion for 4 hours. When the flush subsides the larvae come to the surface, and follow the slowly receding water back to the central pools.

When the flush was stepped up to three times a week, with a double flush imposed from an additional sluice another complication appeared. The bed and banks of the stream started sprouting a heavy growth of vegetation with tufts of grass in mud stream, all of which afforded additional harbourages for *gambiae* larvae. In a canalized bed the results of flushing would probably be much more effective, but it all shows how extraordinarily careful and critical one must be in applying to one kind of mosquito measures which have been established by observations on a different kind of mosquito.

As for *gambiae*'s specialist habits, what is always most striking is not so much the places where *gambiae* does breed, as the great variety of surface water in which it does not breed. Even the ideal breeding place associated with *gambiae* the small sunlit puddle or pool, may be curiously negative. In some of the laterite flats near Freetown, an immense number of such

pools may exist for weeks on end in the rainy season, and yet the proportion breeding *gambiae* may be as low as 1 in 50. But in the odd pool, or group of puddles, which seem to satisfy all requirements, *gambiae* breeding seems to run riot, and hundreds of larvae and pupae may be taken in a few square feet of surface water. The existence of these odd pools swarming with larvae has been one of Freetown's problems since the time of Ross (Ross *et al*, 1902, Ross, 1902). In Lagos, too, I have seen a single flooded vegetable garden in the heart of the densely packed town, whose output was worked out at about 50,000 female *gambiae* per day for several weeks on end.

This feature of *gambiae* breeding is one that crops up on the whole coast, and probably in the whole of West Africa. In this respect I think that during the last few years there has been an increasing realization that the most rapid and striking reduction in the numbers of *gambiae* can be brought about, not by large and costly drainage schemes, but by the so-called temporary measures, systematic search for, treatment or elimination of the small pools and puddles which form the major breeding places. There are many places where breeding grounds have been greatly reduced or eliminated by well planned drainage schemes, there are places like Lagos Island where improved drainage, and the slow and inconspicuous process of sand filling over many years, have produced a tropical swamp town almost completely free of *gambiae* breeding places. But unless these schemes are coupled with an efficient organization for routine inspection and treatment of casual pools, their good effects may be completely nullified.

These remarks naturally do not apply to *melas*, whose control, as we have seen, is an entirely different problem, in which large scale bunding or other engineering schemes is the only answer.

Of the measures directed against the adult mosquito regular house spraying with pyrethrum in kerosene has been used a great deal in recent years, particularly in Gold Coast (FODDY 1944). The use of spray-killing alone has been advocated as an effective means of controlling anopheles in West Africa, a suggestion which seems to be supported by the undoubted success of spray-killing against *gambiae* in South Africa, and against *culisfacies* in India.

In villages near Lagos however we have been unable even by intensive spraying 4 to 6 days a week, to bring about a substantial reduction either in the number of anopheles or in their infectivity probably due to the finding that in houses which are being frequently sprayed more and more mosquitoes tend to leave the house after feeding and rest outside thus escaping further exposures to day time spraying (MUIRHEAD THOMSON, 1947).

However disappointing the results obtained by this measure alone it may still be possible that when it is combined with vigorous anti-larval work, spray killing might provide an additional effective blow against the few remaining adults.

In this respect it is important to remember that the Rockefeller Foundation and the Brazilian Health Authorities in their outstandingly successful

campaign against *gambiae* in Brazil, emphasized the importance of attacking both the larvae and the adult mosquito.

In this report the emphasis has been on *gambiae* and *melas*. No mention has yet been made of *funestus* which is *gambiae*'s able partner in crime over so much of tropical Africa. Actually in the West African coastal strip, on which the colonial capitals are situated, *funestus* is only of local importance. Where it does appear it has not been neglected. For example in Kiley village, now almost a suburb of Freetown, *funestus* used to be as important as *gambiae* (GORDON *et al.*, 1932), but in the last few years, as a result of measures recommended by BLACKLOCK and WILSON (1942) *funestus* has now dwindled to practically nothing.

*Funestus* was also the subject of some of the earliest field experiments on the larvicidal effect of DDT carried out by Professor Buxton (1945) near Takoradi over 2 years ago and RIMANDE (1946) has also added to our knowledge of this species by observations in Gold Coast on the effect of bush clearing, moonlight, and other factors on nocturnal activity.

There are odd localities, too, where *A. nish* has been shown to be an important vector. But in general *gambiae* and *melas* have received the lion's share of attention, and rightly so.

#### DDT IN MALARIA CONTROL

No discussion on recent knowledge of malaria control would be complete without at least passing reference to DDT.

DDT needs no introduction here: the main features have been fully dealt with by Professor Buxton (1945) in his address to this society and in the discussion that followed. While its remarkable insecticidal properties are firmly established there are one or two instances where it has been acclaimed in a way hardly justified by the available facts.

As a larvicide there is no doubt that it is a most valuable weapon. A report from Freetown by Dr. WATSON says that with 2 oz. DDT per acre, using a watery dilution of a kerosene soap-sol emulsion a complete kill of anopheline and culicine larvae is obtained. To achieve the same results with Paris green cost three to four times as much, and about twenty-five times more in the case of malanil. In this way DDT is certainly going to improve control without necessarily revolutionizing it.

Now we come to the question of DDT as a residual insecticide in houses. The commonly accepted idea is that treatment of the inner walls and ceilings of village houses will make them lethal to mosquitoes for two or possibly more months. This conclusion is supported by reports from many countries, and dealing with different anophelines. If this were indeed true, then it would undoubtedly open up entirely new vistas of mosquito eradication and malaria control.

Unfortunately, in some recent work in Lagos, we have disclosed unforeseen snags which may greatly limit the value of this insecticide against mosquitoes. This is briefly the story. When one sprays the walls of village houses with DDT in kerosene, there is the most dramatic fall in the number of mosquitoes caught resting there by day, and this may continue for several weeks or even months. That is the sort of experiment that has been done in many different parts of the world, and many people have taken that as sufficient proof of mosquito reduction.

In Lagos, after a careful study of the normal house haunting habits of *gambiae* and *melas*, we found that although many *gambiae* and *melas* remain in dark houses after feeding at night others leave the house at dawn and rest outside. When they leave the house at any time between dusk and dawn they are attracted by the faint light coming in at windows and other openings from outside. We designed a simple trap based on this attraction to faint light in such a way that all mosquitoes leaving the house were trapped in a small detachable window cage. By this means we were able to show that while a house treated with DDT in kerosene remains free of resting anopheles for weeks afterwards, as early as the 4th day after treatment mosquitoes were entering the hut, feeding on the occupants and leaving the hut after feeding. Of those mosquitoes escaping from the treated hut in this way, and caught in the window cage, there was no appreciable mortality in the following 48 hours. Within a week of treatment large numbers of mosquitoes were feeding in the hut every night, all of them leaving afterwards, so that nothing was found in the hut on the following day (MUIRHEAD THOMSON, 1947).

Presumably what is happening is that when blood-fed anopheles want to settle on the walls after feeding, they are irritated by slight contact with the treated surface, and quickly leave the hut before they have absorbed a lethal dose of the insecticide. We could find no evidence that mosquitoes were succumbing to the effects of insecticide inside the hut.

In another experiment, an isolated village had all the houses treated with DDT in kerosene, but we continued to find blood-fed anopheles in outside resting places beside the village, and among those mosquitoes with gland infections.

This irritant quality of the DDT in kerosene, which prevents it exercising its full lethal action, may possibly be overcome by using DDT in other forms, or it may be influenced by the type of house or wall surface. But at present it may prove a serious obstacle, and there is certainly no cause as yet for undue optimism, in West African conditions at least, about revolutionary results of residual spraying with DDT in kerosene.

We cannot say yet whether or not these findings have any bearing at all on the use of DDT against mosquitoes in houses in other parts of the world, but it is fairly clear that the dramatic fall in house catch following treatment with DDT as a residual spray in houses, cannot be accepted as definite proof

that mosquitoes are being killed. The methods used in West Africa might be applied with advantage elsewhere to follow up the possibility of mosquitoes feeding in treated houses, and leaving unharmed.

If there are any differences of opinion at this stage about the residual effects of DDT on mosquitoes in houses I think that the field entomologists themselves must share the blame. There is little doubt that the sudden advent of DDT with its remarkable residual insecticidal properties caught us unprepared. Our knowledge about mosquito movements in houses was not nearly adequate enough to provide fool-proof field tests. The work in Lagos has shown that without a sound knowledge of the mosquito's normal behaviour it may be very difficult to interpret accurately the results of field tests. The work is really just beginning, and it is still just as necessary as ever to approach the subject with the same caution exercised by Professor Buxton in his address to this society nearly 2 years ago.

#### ANOPHELINE REDUCTION AND MALARIA CONTROL

In this mainly entomological report no mention has been made of the effects of all these measures on the incidence of malaria, particularly among the Services, during the last 5 or 6 years. This itself is a very big subject and one which I am not competent to review or discuss. I understand also that this aspect of the problem will be dealt with fully in a forthcoming publication by Dr TREDDY.

We usually refer to our measures to reduce anophelines as anti-malarial. But in West Africa we do not know at what point the two terms become more or less synonymous. Everything depends on whose malaria we are trying to reduce, non-immune European, or indigenous African.

In this connection I should like to mention some interesting work that has been going on in Freetown for the last 3 years under Dr TURNER and Dr WALTON (TURNER and WALTON 1946; WALTON 1947). They have been concerned with finding the relation between the great reduction in anophelines which they have brought about in Freetown over that period, the extent to which malaria transmission has been reduced, and the extent to which malaria rates and the blood picture of the local African population has changed. Noteworthy is the regular blood examination every month where possible of infants from birth onwards to 18 months, so that exact data are being obtained about the frequency of new infection and their seasonal distribution.

It is obvious that a great deal more information of this kind is required. Even the entomological specialist, narrow though his aim and outlook may be, can hardly help noticing that in these coastal areas at least the indigenous African seems hardly aware of malaria. Such, I do not think anyone is very sure about the extent to which malaria contributes to the ill-health of the adult West Africa, and one naturally comes up against the controversial issue as to how far and to what extent measures against anophelines in West Africa

should be extended beyond the large towns and ports and main centres of non immune population. It seems to me that before we talk loosely about rural malaria control in West Africa it would be a reasonable idea to find out first exactly how important rural malaria really is in that part of the world

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## DISCUSSION

Professor P A Buxton MR PRESIDENT, I should like first to thank our speaker and congratulate him on having delivered an admirable paper which will be of great service to us, and for having spoken so briefly. Dr MUIRHEAD THOMSON has delivered the goods, closed and sat down in 45 minutes, a most unusual accomplishment. There is truly a remarkable amount of information that has come in from West Africa during the last 4 or 5 years



I have been frequently in that country and know the amount of hard work needed to produce this communication and summarize it. One point of considerable interest is that after THOMSON's first period of service in Asia, the Colonial Office was moved to offer him an appointment in West Africa, with the general mandate that he should go there to study the biology of *gambiae*, not to control it, but to contribute to our knowledge of that one species of insect. He has been there 4 or 5 years concentrating on *gambiae* and *melas* and very clearly this policy of concentrating on the main vector but leaving the man to develop the subject in his own way was sound. The decision showed vision on the part of the Colonial Office, and has in fact proved sensible and wise. In the paper there are a great many things that are distinct and valuable contributions to the subject. First of all Dr MUIRHEAD THOMSON's own discovery that you can separate *melas* and *gambiae* apparently every time by the character of the eggs. Every previously known character of adult and larvae were less satisfactory. Having found this point of distinction, another very valuable advance was the discovery of the association of *melas* breeding with the orchards of *Avicennia* in Freetown estuary. If you read that story at length you will find it a fascinating account of most careful natural history. The distribution of the different sorts of mangrove depends very precisely on their position on the very gradual slope in the estuary. Just a bit down the hill you will have the red mangrove, *Rhizophora*, harmless from the mosquito point of view and a little up the slope you may have the *Avicennia*. The elaborate story of the relation of the mosquitoes not only to the mangrove but to a particular point in the tidal cycle is a convincingly worked out story. A third point that will stand out for a long time in the paper and in the work done in West Africa, is the doubt thrown on DDT for this particular purpose against *gambiae* under West African conditions. It is noteworthy that within the last year Dr MUIRHEAD THOMSON and at least one other penetrating worker have begun to feel considerable doubt as to whether DDT is as effective in killing adult mosquitoes in houses as has been believed. I agree that Dr THOMSON's full paper shows that under his conditions DDT is acting more as an irritant than as an insecticide. It is driving mosquitoes out of the house, not to die but to live, and some to develop sporozoites, which after all is the one thing we want them not to do. They are living long enough to become harmful, and that is a very disappointing and disturbing thing. It seems to me that most other users of DDT have failed to look far enough, or deep enough, and have been satisfied with the more superficial observation that after putting DDT on the wall you did not find mosquitoes there for 3 months. There is, however, evidence for the sort of *Anopheles* found in the Mediterranean and in the southern parts of North America, that they sit on walls treated with DDT and remain long enough to take up a lethal dose. Perhaps there is a difference between the different species of *Anopheles* into which further and fuller enquiry will have to be made.

## DISCUSSION

**Dr C R Ribbands** Mr PRESIDENT, there is very little that one can add to the excellent paper that Dr MUIRHEAD THOMSON has given us. I think the thing that must be interesting us all at the moment is not the biology of *melas*, fascinating as it is, but Dr MUIRHEAD THOMSON's most important work on the effect of DDT on *gambiae*, and his critical comments on the effects of pyrethrum spraying. In this connection the first thing I would like to say concerns repellency. Dr MUIRHEAD THOMSON found in West Africa that after pyrethrum spraying mosquitoes came into the room that had been sprayed and then left again. In somewhat similar work, conducted in Assam, I found that *minimus* were not there after pyrethrum spraying, and further work in the evening showed that they did not come in at all. It seems to me that it all depends on whether you offer them an alternative place or not. In my experiment the mosquitoes had the alternative of going into the adjacent houses which were not pyrethrum-sprayed, and they took it, whereas in Dr MUIRHEAD THOMSON's case after spraying they had no choice, and so they went into the sprayed houses to feed, and afterwards came out again. I think that a rather important point when considering the protection of individual houses. One often finds places where there is a mixed population of immune local people and non-immune whites, and one is very much interested in protecting the small local population of whites. In that case it is quite possible that one can get some definite malaria protection by spraying individual houses before or during the evening in order to get the effect of repellency.

Dr MUIRHEAD THOMSON's fundamental work with DDT in the field seems to lead to the conclusion that residual sprays of DDT are almost useless against *A. gambiae*, but those of us who have worked with it elsewhere are not quite so pessimistic. I agree that repellency of some sort plays a big part in the effect of DDT. I found, in Assam, that treatment of whole tea estates with doses adequate to eliminate mosquito catches often yielded no apparent malaria reduction. That tends to confirm Dr MUIRHEAD THOMSON's point of view. But my heaviest treatments with DDT did seem to produce good effects. I think that the possibility of getting malarial control with residual DDT is reduced, but not necessarily eliminated, by the repellency. It may be possible to apply a dose so big that mosquitoes alighting will immediately absorb a lethal dose. If that is possible we may still get malaria control by DDT with massive doses, although it may be a failure in small doses. The trouble is that massive doses are expensive, and if we have to use massive doses of DDT to get malaria control we shall have to specialize and put it only in certain parts of rooms or in places where mosquitoes are most likely to alight, instead of spilling it all over the walls and ceilings according to the present standard method.

**Dr J W Scharif** I desire also to pay hearty tribute to the vivid eloquence of the speaker. The remarks I wish to make must inevitably be coloured with memories of similar work carried out in Malaya. I am particularly pleased to

hear how it has been possible to amalgamate agricultural land improvement with the anti larval work in some of the anti malarial coastal drainage measures described by Dr MUTIRHEAD THOMSON. Before the war in Malaya practically all rural anti malarial projects, including those along the coast, had been associated with land reclamation and were carried out in such a way as to provide additional acreage for crops. If the question of extending anti larval measures into the rural areas of West Africa comes to be seriously considered it should, I submit, be done with very close regard for any opportunity this may offer for the extension of land cultivation and crop production. It had been found in Malaya that relatively inexpensive anti-malaria drainage schemes could be designed to facilitate agriculture. The modern generation of malaria field workers should appreciate the necessity of growing food as well as of arresting disease. Present-day needs have shown how necessary it is that these subjects should be closely allied.

On the question of malaria immunity though one has often heard the view expressed that such an immunity amongst African natives implies no crippling effect among the infected community yet experiences in other equally malarious countries, where the difficulties and tragedies entailed in the build-up of a solid immunity may have been more closely observed, suggest that a light-hearted acceptance of the burden of malaria, even in rural Africa, may not be wholly justified, when "health" is the final goal.

I should like to congratulate Dr THOMSON on his choice of the term "direct," in place of the much misunderstood epithet of "temporary" used in relation to anti malaria measures. In Malaya we were driven to the use of the term "semi permanent" when we were in fact employing methods which were direct and permanent, but which were not so expensive as brick and masonry to which the name "permanent" is generally assigned.

I find it difficult to understand the failure of sluicing mentioned in the paper now under discussion. Is it not possible that the larvae were breeding in the impounded water above the dam, as was described by Dr P F RUSSELL in the Philippines? Then there is a point, which I understand will be further elaborated by Professor WILLIAMSON and which I believe to be of much significance. Dr THOMSON spoke of the remarkable specificity of certain of the breeding haunts of the larvae of *A. gambia* and this contrasts sharply with what one has previously heard of the almost universal nature of its breeding habits. This point of specificity in the breeding places of the malaria-carrying anopheline is one of great importance to field workers and is one wherein much more detailed chemical, geological and biological investigation should be done to determine the underlying characters of the favoured breeding places. The sensitivity of larvae to soil conditions underneath their breeding water is a direction in which one may hope to enhance the value and scope of malaria field surveys. Malayan experience points to the fact that the character of the soil and possibly of the soil bacteria circulating in water in contact with such

soil, may have some effect in determining whether the anopheles which breeds there, is likely to become readily infected with malaria

Finally with regard to DDT, I had the opportunity to assist in some of the early experiments on air and ground spraying with DDT during the war years in India and Burma. In the course of this investigation we tried to determine the relative values of indoor and outdoor spraying under a variety of conditions, but the report of these trials remains buried in the secret archives of South-East Asia Command. The R A F Station at Cuttock, near Calcutta, lent itself remarkably well for some of these tests. On the outskirts of the aerodrome there were some huts identical in character and well stocked each night with an unusually wide range of different species of *Anopheles*. In the particular experiment I have in mind, one hut was sprayed with DDT in kerosene at the rate of 20 mg per square foot and another remained unsprayed. Both the treated and the untreated huts were closed with nets every morning after sunrise and counts were made of the mosquitoes caught over a period of 2 months, during the height of the malaria season. In the course of this trial no live mosquitoes were caught in the hut treated with DDT. The condition of the building made it possible to collect dead mosquitoes found lying on the cement floor as well as on the window ledges and hanging on cobwebs. The findings were remarkable not only because of the long lasting effect and the wholesale slaughter of these insects, but also because of the unending supply of dead anophelines which could be identified, thus revealing the number of different species which had succumbed to the effects of the poison. The total number of the dead catch was on some occasions higher than the numbers caught alive in the untreated hut, care was taken that none was left uncaught. Many of the anophelines not ordinarily house-haunters were found dead and from this it was assumed that, after feeding upon the animals stabled in these huts, they must have been impelled by reason of their engorgement to rest on the DDT-treated walls to extrude the blood plasma before flying away and that the period of resting was long enough for them to acquire a fatal dose of poison. This was the sort of evidence upon which the comforting conclusion was reached that indoor spraying with DDT would probably be successful elsewhere if applied with the same thoroughness as was done in the experiment. It is therefore to be hoped that more detailed study of the value of indoor spraying in West Africa may be made to determine the precise conditions under which the elusive malaria vector, the habits of which have been so charmingly described by our speaker this evening, may be killed by DDT.

**Professor K B Williamson** I have listened with great interest to Dr MUIRHEAD THOMSON's account of his illuminating investigations on the anopheline vectors of malaria in West Africa, and I should like to refer very briefly to a question that concerns this and all similar investigations, namely, what makes one species of mosquito at one time and place an effective carrier of human

malaria, but a poor carrier or absolute non vector at other times, or in other places? I believe it is customary to explain this fact by reference to "species sanitation" in its varietal aspect, the proof being considered complete when it is shown that certain varieties within the species are predominantly non-anthropophilic. The case of *Anopheles maculipennis* provides the best investigated example. It is obvious that the biting of man is as necessary for malaria transmission as turning the switch is for producing electric light. But this may not be the whole story. The mere making of contact will not ensure these results if there is no current to flow nor parasite present to pass on. Some soils seem to bring this about by contaminating breeding waters, especially water logged alluvial soils rich in rotting vegetation. Sir RONALD ROSS counselled investigation of the relations of soil and malaria by modern methods, but I am not aware that much interest has been taken in the subject. Does contamination brought about by organically rich soil underlying stagnant breeding waters, or by excess of vegetation rotting within them directly diminish or abolish the vectorial power of mosquitoes adapted to them, by rendering their tissues biochemically unsuitable for development of the malaria parasites? Be that as it may I think Dr SCHAEFF will bear out, and I believe Sir MALCOLM WATSON and Dr BARROWMAN would if they were present, that Malayan experience shows that the incidence of malaria is largely determined by soil characteristics. Sir MALCOLM WATSON reached the conclusion that dangerous mosquitoes were absent from certain coastal ricefields because of the chemical composition of their water. And subsequent investigation showed that a large area free from *Anopheles* and almost devoid of mosquito breeding stagnated over stale water logged peat. And there is very little malaria in the western coastal rice belt of Malaya, the soil being an ill-drained alluvium rich in rotting vegetation. The coastal forests provide an intriguing exception, but the malaria rate rises steadily and the soils get purer and lighter and are better drained and carry purer water the higher one goes inland. This relationship is not by any means confined to Malaya. COVELL's world lists contain too many examples of destructive vectors which breed in spring-fed seepages, clear streams, rock pools, etc., for the correlation between purity of soil and water and malaria to be entirely accidental. Examples could be multiplied. I learned in conversation with Dr MOREAU of a locality in French Indo-China where *A. vagus*, elsewhere a harmless puddle etc., breeder had been proved to be a vector of malaria, and it turned out that it was breeding on a sandy river bank. Dr McARTHUR, who is present tonight, and who has made the important discovery that *A. leucophrys* is a malarial vector in British North Borneo tells me that he inclines to the belief that there may be two varieties, of which one only breeds in spring and seepage fed water and carries malaria, while the other so far as is yet known does neither.

As is well known, contaminated food and environment contribute flavour to fish, a liberal dressing of Worcester sauce being necessary to render palatable those caught in muddy waters. The same thing occurs in mosquitoes, as an

observation made in my laboratory by ZAIN showed An adult *A barbirostris*, bred from a surviving full-grown larva kept for a few days in dark brown water in which excess of lawn clippings had been rotted, had its midgut and Malpighian tubes stained a deep black, presumably by chemical transformation of the refractory humic matter present in the water, under conditions of defective oxidation approximating to those in waterlogged organic soils One hesitates to conclude that delicate malaria parasites could undergo development on tissues so highly charged Much less contamination might suffice to prevent its completion Only experiment can decide the issue, and in no controversial spirit I venture the suggestion that the problem of the ecological determinants of vectorial power merits investigation at the highest biochemical level by means of modern microchemical technique

If Dr MUIRHEAD THOMSON, repeating experimental enterprise that reaped important discoveries from the miracle of his "aphrodisiac" lampshade, can find or make time to carry out such work, this Society and science will be yet further indebted to him

**Dr G A Walton** While in Freetown I had an ample opportunity of seeing the ways in which one found *A gambiae* larvae in particular places The soil may be mentioned The whole of this area is derived from acid igneous rocks The water is very acid, so much so that even when stream water in Freetown contains large quantities of ammonia the pH is still on the acid side It would be very difficult to alter the soil because there is such a colossal acid reserve nevertheless, I think an investigation into the reason why *A gambiae* breeds in certain places is really called for I wished when I was there that I could have the opportunity to do so, but we were all very busy getting rid of *A gambiae* itself It is worth while mentioning that in my opinion this anopheles breeds in places that are definitely associated with human activity It is not exactly very easy to explain that, but you may have a very large field with an enormous number of small pools in it, and the ones that *A gambiae* chooses to lay eggs in are the ones recently formed by workmen who have been getting rock out of the ground Another thing worth mentioning is that the *A gambiae* seems to show a preference for places where people wash in or cross streams Also in swamps it chooses the places where people walk through the swamp We have also found *A gambiae* breeding in a font in a church! and in one or two other curious places In general the impression is that some form of human interference definitely excites the *A gambiae* to the place where it will lay eggs It is a very interesting problem indeed, and I think something could be done there really valuable

**Dr Edward Hindle** MR PRESIDENT, I should like to take the opportunity of saying what a very special pleasure it has given me to be here this evening and listen to the address by my first Honours Graduate at Glasgow It is also a pleasure to see the result of a zoological training in problems of this kind

Dr MUIRHEAD THOMSON has brought to our notice several observations of great interest in general biology apart from their special application in the problem that he has attempted to solve. There are one or two questions I should like to ask him. One is whether any observations have been made to determine the factors governing the selection by the mosquito of any particular site for the laying of its eggs. I mention this because another of my students, Mr STUART studied the egg laying of different species of Chironomids breeding on the shores of Millport, which are extraordinarily particular in their selection of pools. These pools contain varying mixtures of fresh water and sea water even though they may be only a few inches apart, and you get larvae of one species of Chironomid in pools of a particular salinity whilst adjacent pools of different salinity contain larvae of other species. It is not a question of survival, because you can take larvae from one pool and put them into another of different salinity and they will survive and complete their development. What causes each particular pool to contain only certain species was not determined, but evidence was obtained that the females selected pools according to the salinity of the water. It was not a question of any difference in the pH value. With reference to DDT the thing to study would seem to be the tarsi of anophelines, since this is the part of the body which comes in contact with the insecticide. I imagine that the structure of these tarsi must differ considerably and perhaps the thickness of cuticle or some such factor might help to account for the varying results obtained with different species. In conclusion, I should like to express my thanks to the lecturer for a very interesting paper.

Dr John McArthur I have no real contribution to make to this very delightful paper but our observations in Borneo suggest that results there with DDT would be parallel to those of Dr THOMSON in Africa. The Japanese occupation of Borneo has prevented our trying out spraying of walls there with DDT but, while this of course must be a matter of experiment, it is difficult to believe that it would be lethal to our vector mosquito which enters the houses late at night, feeds on the sleeping natives and returns to the jungle again apparently without resting to any extent on the walls.

Dr R Ford Tredre I should like to say what a pleasure it is to me to have heard Dr MUIRHEAD THOMSON speak to this Society because directly and indirectly I have been associated with him during the time he has been doing this work. When in the R.A.M.C. I was posted as a malariologist to West Africa, and reached Freetown within a couple of months of Dr MUIRHEAD THOMSON's arrival. I may say that I went to West Africa with the idea that malaria control from the military aspect merely amounted to control of fresh-water *gambiae* but when I got there I was rather surprised to find that this brackish water form might be of importance. There was no reliable information obtainable in Freetown as to the importance of *mexes* as a vector of malaria along the shores of the estuary. Major RIBBANDS unfortunately for me, was

## DISCUSSION

transferred to another theatre of war almost immediately and took leave without an entomologist. The malarialogist is very much at sea without his entomologist, but I soon made contact with Dr MUIRHEAD THOMSON. I found that he was already, within a period of 6 weeks, on the track of this differentiation of *melas* from *gambiae*, and as a result of his very clever work we were able to estimate the density of *melas* in relation to the total *gambiae* population in any one village with which the Services were in contact. Along the southern border of the estuary from west to east there were some six villages. In number one we found the population almost entirely *melas*. Number two (Freetown) and number three (Kissy) were practically free from *melas*. Number four was practically entirely *melas* and so it went on. All measures of control in the field had been directed against fresh-water *gambiae* only, so that quite obviously at that time, it was 4 years ago, Dr MUIRHEAD THOMSON'S work was most valuable. At about this time GILROY, working in Lagos, had commenced "bund" construction, which is still in progress and has had dramatic results in the reduction of the anopheline population. It remains to be seen what effect this reduction in anopheline population will have on malaria incidence.

The President (Dr C. M. Wenyon). If no one else wishes to put questions to Dr MUIRHEAD THOMSON it remains for me to ask him to reply to those which have been raised, but before he does so I should like to ask him two for my own information. He has stated that *Anopheles gambiae* showed a higher infection rate with malaria than did the variety *melas*. Is that difference due to differences in the habits of the two mosquitoes, or is it that the one is more readily infected than the other? The other question relates to *Anopheles gambiae* in Brazil. Has it been shown that the important Brazilian strain was a pure *A. gambiae*, or was there any admixture with the variety *melas*?

Dr Muirhead Thomson (in reply). I do not think it will be possible to reply to all questions individually as it would take too long.

With regard to the question that has just been asked by the President, as to whether there is any difference between the habits of *melas* and *gambiae* to account for their different infectivity, we have some evidence in Lagos that *melas* feeds on other animals to a greater extent than *gambiae*. As to experimental infections, the work of J. D. ROBERTSON a few years ago showed that under experimental conditions *gambiae* and *melas* were about equally susceptible.

The questions put forward by Professor BUXTON, Dr SCHARFF and Dr RIBBANDS about DDT can, I think, be answered all together. The work in West Africa has disclosed some unforeseen snags. No doubt there will be more snags, but I must emphasize that the results as yet only apply to West Africa, and the particular type of village house there. The methods of investigation on the other hand might be applied with advantage elsewhere. I must not forget to add, in reply to Dr SCHARFF'S question, that we could find no evidence at all of any anophelines being killed by DDT inside the houses, although I am



aware that in other countries dead mosquitoes have been found in treated houses. The success of such control measures may depend entirely on differences between species and differences in housing. We are only at the beginning of the problem, and if we approach it with a more thorough preliminary knowledge of the mosquito's normal behaviour it should be easier to interpret some of the field experiments.

Dr RIBBANDS raised a point about the mosquito's reaction to pyrethrum when an alternative untreated house is available. I have some unpublished material about this which I don't think he has seen. In these experiments a group of isolated houses were all sprayed with pyrethrum, so that the mosquitoes had no choice of feeding in untreated houses. Under those conditions just as many mosquitoes as usual fed in the sprayed houses. But if some houses were left untreated, so that mosquitoes had a choice of feeding in sprayed or unsprayed houses there was then a definite fall in the number entering and feeding in the hut sprayed with pyrethrum. In field experiments the results may depend a great deal on whether such a choice of blood meals in untreated houses is available or not.

There is the question by Dr WALTON and Professor WILLIAMSON about the composition of the water in the breeding place. This is a very important point and one which I hope to look into. I have had some experience of investigating this kind of problem in Assam, and think that research of that kind has to be done on a long-term policy but results in the end are well worth while. The main reason for not tackling this question in West Africa is that we have been fully occupied with more immediate practical concerns, but the problem will have to be tackled some day. This selection of some types of water for breeding purposes, and avoidance of others, is not entirely determined by the female mosquito. We may find only one pool in fifty with plenty of eggs present, but that particular pool may not turn out to be the most favourable for breeding, as it may dry up or the eggs be washed away.

When we discovered the egg differences between *melas* and *gambae* we followed their distribution by collecting eggs from fresh- and salt water pools, and found *melas* almost entirely confined to the tidal belt as far as egg laying was concerned. We have odd records from fresh-water pools, but *melas* has a decided preference for salt water. *Gambae* shows an equally distinct preference for fresh water but there is no evidence yet to show why *gambae* selects one particular type of fresh water and not another. We have some ideas about this but nothing definite so far and hope to tackle it seriously some day.

**The President:** It only remains for me to thank Dr MUIRHEAD THOMSON for his very interesting paper. The success of the evening is I think very largely the result of Dr THOMSON's having given us, as Professor BURTON has pointed out, so much information in the space of three-quarters of an hour, an experience which is rather unusual in this Society. It is a precedent which deserves encouragement. Thank you, Dr MUIRHEAD THOMSON.

## COMMUNICATIONS.

### THE TRANSMISSION OF MALARIA IN BORNEO

BY

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#### INTRODUCTORY

Malaria appears to be hyperendemic throughout the greater part of Borneo. Although some parts, especially on the coast, seem to be quite healthy, there are many places where the spleen rate is consistently 100 per cent, and malaria appears to be almost universal throughout the jungle-covered interior.

Before 1939 no full time research had ever been carried out on the vectors of malaria in Borneo nor, consequently, upon rational means for its control. The following pages are an account of work carried out for 3 years under the North Borneo Government just before the Japanese occupation.

Borneo is the third largest island in the world. It is exactly astride the Equator, and is a mountainous, well watered, jungle-covered and fertile land, with a good climate, and, apart from malaria and those diseases which are a result of the general low standard of living, it is not an unhealthy land.

#### THE VECTORS OF MALARIA

When this work was begun, little was known regarding the vectors of malaria in Borneo. It had long been believed and recorded that *Anopheles maculatus*, the most dangerous mosquito in the interior of Malaya, was also the most dangerous mosquito in British Borneo. For the existence of this belief there was a good deal of evidence and upon it anti-malarial measures had frequently been based. SHIRCORE (1937) for example, wrote that "*A. maculatus*, a notorious carrier in Malaya, is widely and typically distributed throughout the areas visited and must be regarded as the chief vector and there was a good deal of other published evidence of the virulence with which this mosquito was credited.

\* Publication of this article—written in 1941—was interrupted by the occupation of Borneo by the Japanese and my internment by them.

I have pleasure in acknowledging the co-operation of the British North Borneo Government and the helpful interest of Sir MALCOLM WATSON, then Director of the Ross Institute, in this work, a full account of which will be published shortly.

In this belief, therefore, the research was begun under the impression that malaria work there would prove to mean simply the application of those measures which had proved so successful in Malaya, but by some more economical means suited to the far less wealthy land of Borneo.

### THE EVIDENCE OF JESSELTON.

Jesselton is a small port on the West Coast of North Borneo. In this town, for 5 months in the early part of 1939 research was carried out on local malaria and mosquitoes. This work at the time appeared fruitless, as it was not possible to follow it to its conclusion, but it later proved to have a quite important place in the picture of Borneo malaria.

An examination of some 1,200 children from all parts of the town, to determine the intensity and distribution of malaria, found only 2.5 per cent. to have enlarged spleens and since these few could all be traced as having recently come from highly malarious areas Jesselton was accepted as a healthy locality with no malarial transmission. Areas only a few miles away however, were found to be highly malarious with spleen rates of 80 per cent. and more.

Next, a larval examination was carried out in the town. This yielded very few anopheles, and seemed to be in keeping with the absence of malaria.

After this, however the larval survey was continued into the hills behind the town, an area which had never been examined before. Here, surprisingly well within striking distance of the town, were found about fifteen streams all breeding anopheles, among which *A. maculatus* occurred in considerable numbers. This led to much speculation as to why *A. maculatus* should be present in close proximity to a town with no malaria transmission. Three possible explanations presented themselves.

1. That the breeding may have been abnormal, since the survey was carried out following a drought.

2. That the hills and jungle may have constituted a barrier to the flight of the mosquito.

3. That *A. maculatus* might be of little importance as a vector.

It was not possible at the time to investigate these possibilities as it was necessary to proceed up country. Later however it was possible to fit the peculiar findings of Jesselton into the still more peculiar picture of malaria in the interior.

### THE EVIDENCE OF TAMBUNAN

Tambunan Valley is an elevated plain in the interior of North Borneo, lying at an altitude of some 2,000 feet. It is about 25 miles from the sea, but separated therefrom by a 6,000-foot mountain range. The plain, some 10 miles long, varying from a quarter to 2 miles broad is well-watered by rivers, mountain streams and irrigation channels, and surrounded by high

jungle-covered hills, which rise abruptly from its margin and climb to summits of 5,000 and 6,000 feet. Paddy cultivation, primitive and irregular, practically covers the plain, and herds of buffalo graze over the remaining land. The climate here is equable, temperature is moderate, and rainfall not too great, with an ill-defined wet and dry season. It is an isolated region, and inaccessible to wheeled traffic, but relatively populous, inhabited by some 6,000 people. The population is fairly static, and there is very little export and import, although some movement takes place from the plain into the neighbouring areas, and to supply estate labour near the coast. The houses, primitive and built of bamboo, are collected into scattered villages, and distributed over the plain and into the hill ravines.

Amongst the people of Tambunan, malaria was said to be intense, alcoholism rife, and standards of living low. This area, therefore, with its malaria and its static population, presented itself as suitable for malaria research.

Here, the first step was to confirm the presence of malaria. This was not difficult. Every report from dispensary dressers, and by any visiting medical man, referred to the intense malaria in Tambunan. The survey by Dr SHIRCORE showed many villages with a spleen rate of 100 per cent, and a rapid and random examination of a few villages for spleens and parasites, soon confirmed the same thing. In this highly malarious area, therefore, work was begun.

#### EXTENSIVE PRELIMINARY LARVAL SURVEY

A preliminary examination of possible breeding places of mosquitoes, revealed the presence of an enormous number and variety of anopheles breeding places distributed widely over the whole plain. Pools, streams, swamps, seepages, paddy fields, irrigation channels, and buffalo wallows—all presented a choice of breeding places, while even the hoof prints of cattle, and the widespread hollow bamboo fences, bred mosquitoes in numbers. The entire plain, in fact, seemed to be one vast anopheles breeding place.

There appeared to be, therefore, every reason why the area should be subject to very intense malaria, and the problem seemed to be rather how to control it, than how to explain it. This, however, was not altogether the case, as it was very soon discovered that it was by no means easy to explain malaria transmission here.

#### INTENSIVE ROUTINE MONTHLY SURVEYS

The next step apart from preliminary map-making and organization, was to determine what species of anopheles were actually present, with, if possible, some assessment of the relative importance of each. This was done by taking an experimental area of about a square mile, which included paddy fields and irrigation channels, streams, swamps, pools, seepages, buffalo wallows, water-

filled bamboos, and water-logged hoof prints. These were situated in some villages, in open country and in jungle, so that every possible variety of breeding place might be examined. The area was carefully surveyed for the breeding of anopheles larvae, and records were kept of the type of breeding place preferred by each species. This survey was repeated every month for 24 months keeping careful records of the breeding habits, in the belief that, when the vector species were incriminated it might be possible to have full information regarding their habits, and an effective strategy might be available for a campaign against them.

It was found that most of the species of anopheles present are very fastidious in their choice of breeding places. Certain species selected conditions which were shunned by others, preferring to die rather than to be compelled to breed in conditions other than their preference.

It was found that twelve species of anopheles were breeding four of them in abundance, and the remaining eight more rarely in the following numbers and proportions out of a total catch of over 44,000 —

SPECIES OF ANOPHELES	TOTAL	Per cent.
<i>A. barbatirostris</i> V.d.Wulp	23,107	= 57
<i>A. kochi</i> Dön.	7,561	= 17
<i>A. maculatus</i> Theo.	6,147	= 14
<i>A. philippinensis</i> Ludl.	4,804	= 11
<i>A. harricari</i> (James)	267	= 1
<i>A. barbatirostris</i> Strickl. & Choud.	79	= 0
<i>A. leucophyrus</i> Dön.	74	= 0
<i>A. sinellatus</i> Theo.	?	} Figures lost during Japanese occupation, but all less than 0.2 per cent.
<i>A. aitheni</i> James	?	
<i>A. aitheni brugolensis</i> Puri	?	
<i>A. aitheni palmatus</i> (Rdwt.)	?	
<i>A. aitheni</i> (var. nov.)	?	

The problem was to determine which of these were vectors, and which were harmless mosquitoes.

*Suspect d Species*—It was assumed that the first species which should be suspected as vectors should be those which were found to be the most widely breeding and those which had been proved to be dangerous elsewhere. For this reason consideration was given chiefly to the first four of this list, mosquitoes which were not only found in numbers in Tambunan, but which had all been found to be vectors of malaria in other places.

The most common anopheles in Tambunan—*A. barbatirostris*—is a vector both to the east and to the west of Borneo in Celebes and in parts of Malaya. Being found in such large numbers in Tambunan, breeding in pools and paddy fields over the entire plain, it had to be under great suspicion. *A. kochi* found breeding generally in dirty water on the plain, is a vector in parts of Sumatra, and required to be considered. The paddy-field breeder *A. philippinensis*, is a vector of malaria in parts of India, and breeding in enormous numbers toward the end of the irrigation season in Tambunan was under considerable suspicion.

*A. maculatus*, although not in great numbers, was found breeding typically in hill seepages and streams in cleared ravines, and on account of its evil reputation in other parts of Borneo, and its dangerous nature in Malaya, was naturally under the greatest suspicion.

Each of the remaining eight anopheles numbered less than 1 per cent, and none was under any suspicion in Tambunan, nor, as far as could be ascertained then, in any other part of the world, and could not therefore be very seriously suspected.

#### INTENSITY AND DISTRIBUTION OF TAMBUNAN MALARIA

A study was next made of the intensity and distribution of malaria in Tambunan. A survey was carried out in twenty-five villages and in a representative area starting in the centre of the plain among the rice fields, working to the jungle-covered hills at the periphery, and up into the inhabited ravines. This included spleen and parasite examinations in some 1,200 children. As a result a peculiar and interesting situation was found.

It was found that villages in the middle of the plain, in the rice fields, and therefore in the situation of the most intense anopheles breeding, were relatively healthy, with spleen rates of only about 20 per cent, but that malaria steadily increased on approaching the surrounding hills, until in the hills and in hill ravines it was 100 per cent, although there was far less anopheles breeding there. There appeared to be no appreciable seasonal variation in malaria throughout the year.

This was taken as evidence that *A. maculatus*, being a hill breeding mosquito, was the vector of malaria. It had to be admitted, however, that it pointed equally to the other hill breeding species *A. karwari*, *A. leucosphyrus*, and *A. atkensi* in its four varieties, as possible vectors. These were all found only in very small numbers in Tambunan, and were not under great suspicion, as they were believed to be harmless in Malaya. It was also evidence that *A. barbirostris*, *A. kochi*, and *A. philippinensis*, being plain breeders, were of little significance, as they were all found in greatest numbers where malaria was slight.

This was an excellent illustration of the fact that water is by no means necessarily dangerous in malaria, that, in fact, the vast areas of water covering the plain, the paddy fields and irrigation channels, pools, streams, seepages, buffalo wallows, wells and swamps, were at least relatively harmless, whereas the much smaller areas of water in the hills, the seepages, springs, and rapidly flowing streams generally too swift for breeding, were for some reason more dangerous.

Thus, it was accepted as a rule, that the healthiest villages were those farthest from the hills, and the most malarious were those in the hills.

*Exceptions*—To this apparent rule there was one peculiar exception, for which no explanation could for long be found. To the north-west of Tam-

bunan Plain there was a ravine typical except that it was more open than most. In this ravine there were two villages—Sunsurum and Tantalob—and in accordance with the rule it was estimated that they would both be highly malarious, probably with a spleen rate of 100 per cent. An examination found that Tantalob had, in fact a spleen rate of 100 per cent., but an examination of Sunsurum, less than a mile away and within sight of it, yielded, surprisingly a spleen rate of only 25 per cent.

Several attempts were made to explain this. It was assumed that probably there were fewer mosquitoes in Sunsurum but it was soon clear that there were actually more mosquitoes breeding there than in the malarious villages. It was then assumed that probably there were fewer *A. maculatus* than in other ravines but a survey showed that there was, in fact, more *A. maculatus* breeding, and that recent clearing had encouraged this rather than otherwise.

A search of the records showed that, 10 years before, this village had had a high spleen rate of 85 per cent. What had caused the recent fall in malaria, although with a rise in *A. maculatus* it was difficult to see. Further surveys in the vicinity of Sunsurum only deepened the mystery for the village was found to be in close proximity to cleared ravines of a type which in Malaya which would be considered very dangerous and the ravines were in fact breeding *A. maculatus* in considerable numbers, to the very doors of the houses.

This anomaly was a constant challenge to conservative thinking but ultimately it proved the key to the problem of malaria in Tambunan and, in fact, throughout at least a large part of Borneo.

#### ADULT MOSQUITOES.

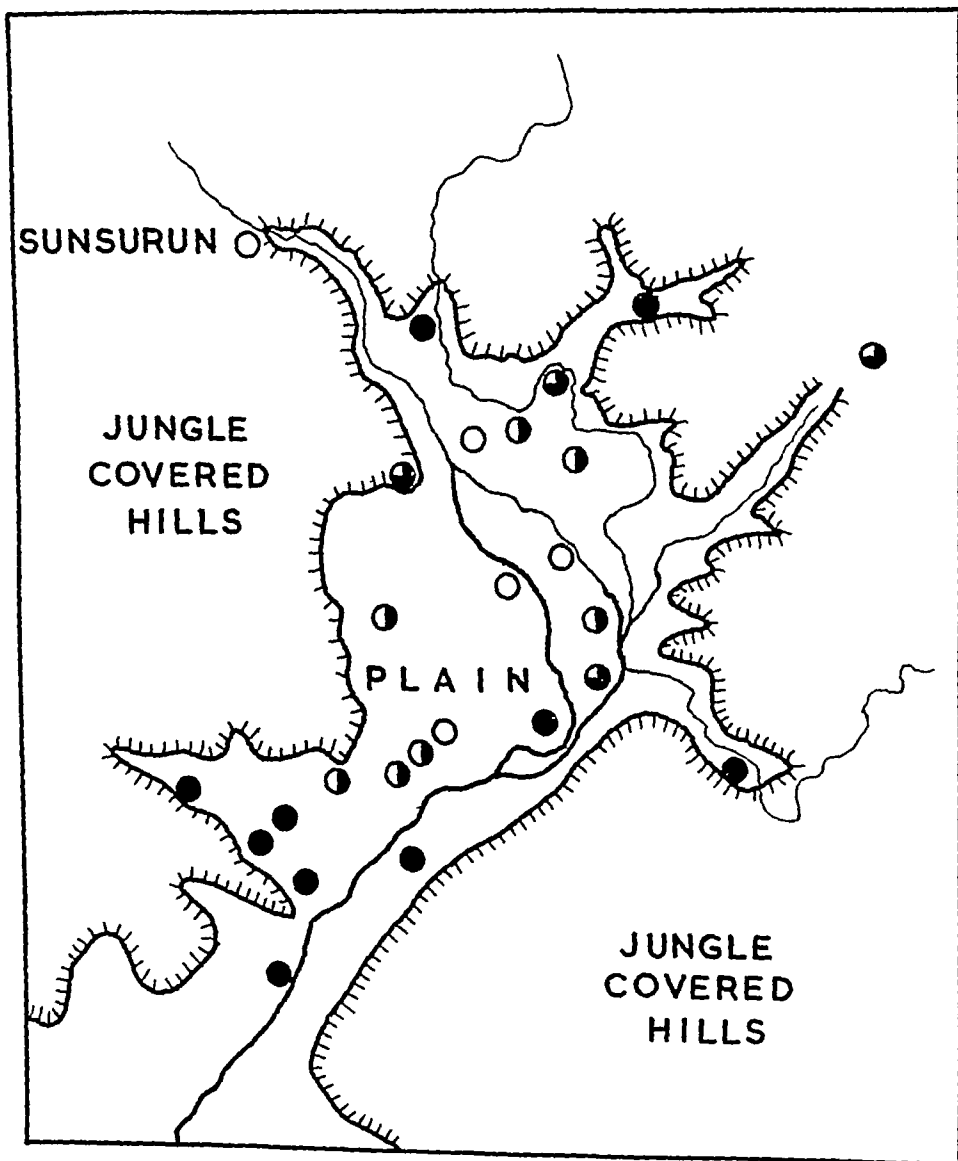
Although valuable information may sometimes be obtained in this way from the evidence of larval breeding, this is only indirect evidence, and cannot be accepted as proof. The only absolute proof that a species is responsible for transmitting malaria is by the finding, on dissection, of a proportion of adult mosquitoes infected with malaria parasites. Adult mosquitoes were, therefore, sought for dissection, and it was proposed to dissect at least 100 and if possible 500 of every species in the locality.

Here, too, a puzzling situation presented itself because searches for mosquitoes failed to find any and the natives declared that there were none.

*House Searches*—In Africa, India, Malaya, and in fact in most countries in the tropics, numbers of anophelids can generally be captured settling on the walls inside houses during the evening. In Tambunan, however no anophelids were ever seen either during the day or in the course of the evening. Careful searches in every possible type of house in native huts, bungalows, cow-sheds, hen-houses, and elsewhere in every kind of situation by day and night, aided by the disturbance of the furniture and spraying with Flit on literally hundreds of occasions, yielded not a single mosquito either resting or attempting to feed and the natives even in the most malarious villages were insistent

## TAMBUNAN PLAIN SPLEEN RATES, 1939-40

Showing distribution of villages, healthy on the plain and malarious in the hills, and the one important exception to the rule - Sunsurun



SPLEEN RATES 0 - 25 % ○  
 " 25 - 50 ●

SPLEEN RATES 50 - 75 % ●  
 " 75 - 100 ●



that they were not bitten by mosquitoes there. Dr SHIRCORE found the same thing during his survey in 1936. During several month assiduous and skilful search, he saw only one anopheles in a native hut.

The picture presented itself therefore, of an intensely malarious area, with widespread breeding of anopheles, but with apparently no adult mosquitoes to account for the transmission of the disease. Consequently it was difficult to explain how malaria transmission was being maintained and it was impossible to carry out any dissections.

*Human Bait Trapping*—In other parts of the world a human bait trap may be a valuable means of capturing mosquitoes. This consists essentially of an inner net to protect the operator who acts as bait, and a larger outer net provided with an opening which can be rapidly closed for capturing anopheles which are attempting to feed. It is often used in Malaya and elsewhere, and to obtain dissection material in Tambunan therefore such a trap was made and operated in a variety of situations in healthy villages and in the most highly malarious in empty houses and in houses full of people in houses with cases of malaria and where children had died of the disease in the middle of villages, and in solitary huts and open verandahs. It was operated every hour all through the night from sunset to sunrise on hundred of nights, during every season and in every variety of situation.

The results were astonishing. In contrast to other countries, practically no anopheles were ever seen. On most nights nothing was taken whatever during the entire night. On an average only one anopheles was taken during every 15 hours of work. On an average only one *A. maculatus* was taken every 3 weeks after more than 200 hours of work. In one group of highly malarious villages for example with spleen rates of 100 per cent., all night trapping, every night, for sixteen night resulted only in the capture of one harmless anopheles. Such a paucity of material made dissections an impossibility. It was calculated that to obtain the desired 500 dissections of *A. maculatus* it would require an attendant to operate the trap hourly all through the night, from dusk to dawn, 7 days a week for 25 years.

The trap was carefully checked with recording alarm clock, running commentary by the operator periodical visit and occasional personal operation, and there was no doubt about the reliability of the results. Comparison with the operation of a similar trap in Malaya showed that no error lay in the technique.

The most common species taken, though that only rarely was the pool-breeding *A. kochi* generally of little importance, but a vector in some countries next was *A. leucosphyrus* which in Malaya is welcomed rather than otherwise since it is not only considered harmless, but its presence indicates the absence of *A. maculatus*. Present also and during the paddy season by far the most common was *A. phibppinensis*.

Thus the results of human bait trapping did not yield any convincing evidence, and an analysis of the figures simply deepened the mystery. More anopheles were taken, as a rule in healthy villages than in unhealthy, although still in astonishingly small numbers. More *A. maculatus* were taken in healthy villages than in the most malarious. There was no indication of a seasonal increase over the course of 2 years' trapping, and no information of any value seemed to accrue from the different situations of the trap.

A review of the first year's work left one with the impression of an area of intense malaria, in parts with spleen rates of 100 per cent, and widespread anopheles breeding, but without the adult anopheles to account for it.

Suspicion still rested on *A. maculatus*, although it was difficult to understand how its apparent scarcity could maintain malaria at such an intensity. Suspicion also rested as a result of human bait trapping on *A. kochi* and *A. philippinensis*, although their distribution was against this. To be logical suspicion had also to rest upon *A. leucosphyrus* although this, taken in such minute numbers—only one every three or four nights, found breeding also in very minute numbers and believed to be harmless elsewhere, could not seriously be considered to be carrying malaria and maintaining it at 100 per cent. Dissections, however, continued to be carried out on every anopheles taken and always with negative results.

*Adult Resting Places*—It was clear that, if mosquitoes were breeding widely, they must exist as adults somewhere, and therefore they must feed and rest. Since they did not appear to rest in the houses, nor in such cattle sheds and hen houses as existed it was assumed, therefore, that they must be resting in the jungle or elsewhere outside. Consequently, to obtain further specimens for dissection, and to discover where the mosquitoes rested, search was made out of doors, and it was found that, by beating bushes in jungle and along the banks of streams resting mosquitoes might be started into flight, and if followed carefully they might be captured settling in the grass. This provided considerable dissection material, until it was found that mosquitoes captured in this way were nearly all *A. kochi* or *A. philippinensis*, and when 500 of each of these had been dissected with no positive results the search was abandoned, as not worth further time spent on it.

*Animal Bait Trapping*—The question then arose of where the anopheles, breeding in such enormous numbers, were feeding. There were large numbers of buffaloes on Tambunan Plain, more than one for every inhabitant, in addition to cows, ponies, pigs, and other animals, and it was suspected that these might be providing the blood meals for the adult mosquitoes. Searches were therefore, carried out, by tethering animals and searching these at night with a torch, for feeding anopheles.

This also led at first to disappointment, as only one or two anopheles were

found during several evenings search. In the conviction, however that the mosquitoes found breeding must also be feeding, it was determined to watch the animals all night long, constantly from sundown to sunrise and this led to the discovery that about dusk, enormous numbers of anopheles appeared, to feed on ponies and buffaloes. They fed avidly for quite a short time after sundown, and in an hour or so had practically all disappeared.

This explained where most of the mosquitoes fed, and why the previous animal observations, made at night, had failed. It also provided much more material for dissection, but it did not explain in any way the transmission of malaria from man to man.

*Dissections*.—By this time, therefore *Anopheles kochi* and *A. phippinensis*, by providing adequate negative dissections, were exonerated from suspicion as vectors. Dissections of other species, however also continued to be negative, and when 1,500 mosquitoes, obtained with great effort over the course of nearly 2 years, had all been dissected without positive result, hope began to yield to despair. All the conventional methods, and many unconventional ones, had failed to discover the vector mosquito, or even to suggest the solution to the problem. House searching and human bait trapping had proved useless. Animal bait trapping and jungle searching, although at first promising, yielded no positive result, and the problem of the identity and habits of the vector mosquito remained. One was compelled to consider the possibility of Tambunan malaria being carried in some unusual way for example by a jungle-feeding anopheles or possibly not by an anopheles at all.

#### COMPARISON OF HUMAN AND ANIMAL BAIT TRAPPING.

At this point, the results of past work were considered, not only by themselves but in relation to each other and it was found that, whereas the results of human bait trapping and of animal bait trapping did not, by themselves, yield any information, a comparison of the two yielded information which was of the highest value and which led ultimately to the solution of this curious problem, and to the incrimination of the vector.

A study of 1,300 anopheles captured while they were in search of a blood meal, indicated the following preferences of each species for man and animals —

	HUMAN PER CENT	ANIMAL PER CENT
<i>A. kochi</i>	3	97
<i>A. phippinensis</i>	6	94
<i>A. barbirostris</i>	10	90
<i>A. maculatus</i>	15	85
<i>A. harrisoni</i>	17	83
<i>A. leucophrys</i>	28	12
	—	—
Total	8	95

It was found, firstly, that practically all the species of anopheles present showed a much greater preference for animal blood than for human blood, and could not, therefore, be of any importance in regard to human malaria

It was found, with some surprise, that *A. maculatus*, hitherto regarded as under greatest suspicion, had an interest in human blood in only about 15 per cent. of cases, the remaining 85 per cent, seeking their blood meal on animals

Further, the equally remarkable fact was discovered that of all the anopheles present, only one had a preference for human blood, and that this was *A. leucosphyrus*, hitherto disregarded, and considered to be the most harmless of all insects in Malaya. This mosquito showed a preference for human blood in no less than 88 per cent of dissections

The few precipitin tests carried out completely confirmed this finding, all *A. leucosphyrus* captured being engorged with human blood, and all other species, including *A. maculatus*, with animal blood, although unfortunately it was not possible to obtain sufficient numbers to make conclusive series

Thus, the impression arose that the previously unregarded *A. leucosphyrus* might prove to be the vector, and that the suspected *A. maculatus*, together with all the other mosquitoes breeding in the locality, might be harmless

#### THE EVIDENCE REGARDING *A. leucosphyrus*

With this information, the whole work was reviewed. It was noted that *A. leucosphyrus* is a purely jungle mosquito, and that if it were a vector, and *A. maculatus* harmless, it would explain a number of anomalies. It would explain why Jesselton was healthy. It would explain why the centre of Tambunan Plain was healthy. It would explain why the hills of Tambunan were malarious, and it would explain the anomaly of the village of Sunsurun, 'cleared' of jungle and healthy in spite of the increased breeding of *A. maculatus*.

Against the acceptance of *A. leucosphyrus* as a probable vector, however, was its innocent reputation in Malaya, and, above all, its apparently great rarity in the locality. In the human bait trap, only one was taken, on an average, about every three whole nights of operation, and none captured in this way had ever been found infected. Moreover, of 44,000 larvae taken in the locality, only seventy-three were *A. leucosphyrus*. During most months of the year it was never found breeding at all, and it had never been found consistently in a permanent breeding place.

With the information gained, however, a thorough search was made of the literature, and here the evidence was rather encouraging. It was found, firstly, that although generally regarded as harmless, *A. leucosphyrus*

had been recorded as under suspicion, although on indirect evidence, and together with several other species, as a vector of malaria in at least four places in Borneo. Finally it was found that one *A. leucosphyrus* had actually been found infected on the east coast of Dutch Borneo after a discouraging search for mosquitoes, by GORLANSO (1934), and that in an island off the south-east of Dutch Borneo no less than seven of the same species had been found infected by STOKER (1934).

Thus, the evidence for *A. leucosphyrus* as a vector became stronger although its reputation, and especially its apparently extreme rarity still made it difficult to regard it very seriously.

#### THE INCUBINATION OF THE VECTOR.

With this information a drive was made to capture more *A. leucosphyrus* for dissection. Human bait trapping was pursued vigorously in spite of its poor returns. House examinations were renewed traps were experimented with and possible natural resting-places of anopheles were sought for. Searches were made at nights on the backs of ponies, buffaloes, pigs, and small boys. Payment was offered to the natives for any anopheles captured and a prize was offered for the first infected specimen.

It was this that finally rewarded the search with success. The domestic staff, having been encouraged to use anti-malarial precautions for themselves and their families, began bringing in anopheles taken in defective mosquito nets in spite of the previous protest that there were no mosquitoes. Some times they would bring in ten or fifteen in a morning, and these all proved to be *A. leucosphyrus* thus strongly supporting suspicion.

It was from this source in April 1941 after more than 2 years search for mosquitoes, and after more than 1,500 negative dissections, that the first infected specimen—an *A. leucosphyrus*—was found, proving this species to be a vector.

Following this, the search was continued, and out of nearly 800 dissections on *A. leucosphyrus* obtained in this way twenty-five specimens were found infected, while all other species, including *A. maculatus*, remained consistently negative. Positive dissections were carried out not only in Tambunan, but in two other localities in North Borneo thus showing that it was not limited to Tambunan Plain.

Thus, *A. leucosphyrus* which in Malaya is considered to be one of the most harmless of species, was proved to be the vector and almost certainly the only vector of malaria in Tambunan while *A. maculatus* for long regarded as the chief vector in Borneo, was found to have little if any relation to the disease in the locality.

*Total Dissections*—The following dissections were carried out in the interior and on the west coast of North Borneo during 1940-41—

		NUMBER EXAMINED	POSITIVE GUT	POSITIVE GLAND	POSITIVE TONGUE	PERCENT POSITIVE
1940	Tambunan	1 <i>Iochi</i>	—	—	—	—
		1 <i>A. philippinensis</i>	—	—	—	—
		1 <i>leucosphyrus</i>	—	—	—	—
		1 <i>haricari</i>	—	—	—	—
		1 <i>maculatus</i>	—	—	—	—
		1 <i>A. barbrostris</i>	—	—	—	—
	Purutan	1 <i>leucosphyrus</i>	—	—	—	—
		1 <i>maculatus</i>	—	—	—	—
	Apin Apin	1 <i>leucosphyrus</i>	—	—	—	—
		1 <i>barbrostris</i>	—	—	—	—
		1 <i>I. d.</i>	—	—	—	—
		1 <i>maculatus</i>	—	—	—	—
		1 <i>philippinensis</i>	—	—	—	—
	Jesselton	1 <i>litoralis</i>	—	—	—	—
		1 <i>leucosphyrus</i>	—	—	—	—
		1 <i>philippinensis</i>	—	—	—	—
		1 <i>maculatus</i>	—	—	—	—
		1 <i>A. barbrostris</i>	—	—	—	—
	Mengarai	1 <i>philippinensis</i>	—	—	—	—
		1 <i>Iochi</i>	—	—	—	—
		1 <i>barbrostris</i>	—	—	—	—
		1 <i>litoralis</i>	—	—	—	—
1941	Tambunan	1 <i>leucosphyrus</i>	7	13	10	3.0
		1 <i>barbrostris</i>	—	—	—	—
		1 <i>Iochi</i>	—	—	—	—
		1 <i>philippinensis</i>	—	—	—	—
		1 <i>maculatus</i>	—	—	—	—
		1 <i>A. haricari</i>	—	—	—	—
		1 <i>tessellatus</i>	—	—	—	—
		1 <i>A. barbumbrosus</i>	—	—	—	—
	Apin Apin (June)	1 <i>leucosphyrus</i>	0	2	2	18.2
		1 <i>maculatus</i>	—	—	—	—
	Purutan	1 <i>leucosphyrus</i>	—	1	1	7.7
		1 <i>maculatus</i>	—	—	—	—
	Apin Apin (October)	1 <i>leucosphyrus</i>	1	2	3	20.0
		1 <i>barbrostris</i>	—	—	—	—
		1 <i>philippinensis</i>	—	—	—	—
Total		2,715				

These are summarized as follows—

1 <i>leucosphyrus</i>	761	25 positive = 3.3 per cent
1 <i>A. philippinensis</i>	567	all negative
1 <i>A. Iochi</i>	541	"
1 <i>A. barbrostris</i>	521	"
1 <i>A. maculatus</i>	177	"
1 <i>A. haricari</i>	102	"
1 <i>A. litoralis</i>	31	"
1 <i>A. tessellatus</i>	12	"
1 <i>A. barbumbrosus</i>	3	"
Total	2,715	

*Explanation of Anomalies*.—The discovery of *A. leucosphyrus* as the only vector in Tambunan explained all the difficulties that had arisen with the assumption that *A. maculatus* was the vector. *A. leucosphyrus* is a purely jungle breeder demanding dense shade, in contrast to *A. maculatus* which demands sunlight; and that *A. leucosphyrus* was the vector in Tambunan, explained the peculiar distribution of malaria which was least in the paddy fields and greatest in the hills. It showed that the hills were malarious, not because they were hill but because they were jungle-covered. It explained why Sunsurun highly malarious 10 years previously when under dense jungle was now healthy when jungle had been cleared although *A. maculatus* has been encouraged and it explained why Jesselton was healthy in spite of the presence of heavy *A. maculatus* breeding in contrast to its jungle-covered environs which were highly malarious.

#### THE APPARENT SCARCITY OF *A. leucosphyrus*

With the discovery of *A. leucosphyrus* as the vector of malaria in Tambunan, another problem presented itself. *A. leucosphyrus* appeared to be a very rare mosquito both in the larval and adult stages so rare, in fact, as to be easily missed in an ordinary survey. It was a mosquito which had never been found rearing either by day or night. Unlike other mosquitoes, it was rarely found feeding on animals. It was in the human bait trap only once every 36 hours of work. I have never seen a single specimen of *A. leucosphyrus* at liberty flying, feeding or resting and the natives were equally emphatic that they were not visited by this or any other mosquito.

The apparent absence of all anopheles from the houses was explained very largely by the feeding of most anopheles on cattle but this did not explain the absence of *A. leucosphyrus* which did not often so feed.

Again, in extensive larval surveys only one *A. leucosphyrus* was found in every 5,000 larvae taken, and during most months of the year it was never found at all. It is no disparagement of the work of Dr STURGEON or of other previous observers, to say that in skilled and assiduous searches they never even recorded *A. leucosphyrus* as present in the locality. It appeared to be an area of intense malaria, but in which the only vector seemed to be present in such insignificant numbers as to be incapable of maintaining malaria transmission at all.

It seemed clear that for some peculiar reason this mosquito was being missed both in the larval and adult stages, and searches were therefore directed to the solution of this problem.

*The Elusiveness of A. leucosphyrus Larvae*.—First search was made for *A. leucosphyrus* breeding. Since it was found to be a purely jungle breeder although searches had been virtually negative for nearly 2 years, renewed surveys were made in the area previously selected for routine larval

surveys, but with greater thoroughness, and confining attention to the jungle. The previous breeding places were again examined, searches were made in rock pools, tree holes, water-filled leaves, rapidly flowing streams and other unlikely places.

Finally, these having all proved negative, the jungle itself was felled, and it was this that finally exposed the breeding places. Under dense jungle shade, so dense that access was often difficult or impossible without clearing vegetation, in little seepages right up at the sources of hill streams, breeding places were discovered which for 2 years had been overlooked.

It was found that breeding had been missed for two reasons. Firstly, it had been missed because the work had been too conservative. Larval surveys had been made on conventional lines found to be successful in other countries. Searches had been made particularly for *A. maculatus*, and had been too unenterprising to include the denser jungle as important. Secondly, *A. leucosphyrus* was found to be a very shy larva. It was discovered that it not only readily hid in the sediment at the bottom of the dipper, but that it remained submerged for a very long time, much longer than was the case with other species. It was often timed to remain submerged for more than 5 minutes, and it seemed clear that water had often been rejected from a dipper which might, in fact, have contained *A. leucosphyrus*.

Thus, the rule was made that *A. leucosphyrus* could not be claimed as absent from an area unless jungle had been cleared right to the sources of streams, and unless the dipper had been observed for at least 5 minutes after dipping.

When this was realized, it was discovered that, far from being a rare mosquito, it was in fact quite a common one. Breeding was found in no less than 150 permanent breeding places in this area which had previously been regarded as negative during twenty-four careful routine monthly surveys. Although quite widespread, however, once the type of breeding place was understood it was found to be easily recognizable, as this insect was found breeding almost exclusively in clear spring water in tiny seepages at the sources of streams or along a hillfoot, and always under complete shade. It was concluded, therefore, that the great number and variety of other breeding places in the locality, streams, swamps, paddy fields and irrigation channels, pools, puddles, and the innumerable other collections of water, were ~~not~~ although breeding anopheles freely.

The Elusiveness of *A. leucosphyrus* Adults — It remained to be found where the adult *A. leucosphyrus* fed and rested, and this was found to be so rare. It appeared to be completely absent from the area examined by conventional methods and in the opinion of the author and others completely so in experience with the human host. That it was not



in the houses, however was proved by the fact that it was taken in fair numbers, engorged and trapped in defective mosquito nets in the early morning.

How it was feeding was only discovered by the tedious method of sitting up all night and actually watching for individual mosquitoes and it is to the credit of the staff that they did in fact undertake this successfully. For four nights nothing whatever was seen during 12 hours observation, but on the fifth night it was seen that several mosquitoes—all *A. leucosphyrus*—entered the house long after midnight, fed on their sleeping victims, then after a short rest fled quickly away to their undiscovered hiding-places, presumably in the jungle long before dawn.

Why this mosquito came out on some nights and not on others was never discovered but this late feeding in the early hours of morning long after people were asleep, explained why it was never seen, and why it was apparently absent from areas where it was responsible for intense malaria. Further it was seen that, when searching for a blood meal, if *A. leucosphyrus* entered a human bait trap, unlike other anophelids it did not wait long enough to get caught. This explained its relative rarity in the trap. It was observed to be present and feeding on sleeping natives in houses where the human bait trap was being operated unsuccessfully.

Thus this dangerous mosquito although apparently rare and almost absent was in fact quite common, and was being missed because of its elusive breeding under dense and often impenetrable jungle, and of its feeding in the early hours when everyone was asleep and not resting on the walls for any length of time.

The Importance of *A. leucosphyrus* in Borneo.—Tambunan district represents a relatively small part of Borneo, and the findings of one locality cannot be applied without question throughout the whole of the island, so that further work was necessary to define its importance elsewhere.

Positive dissections were carried out in two other places in the interior of North Borneo, Pututan and Apin Apin, finding natural infection indices of 8 per cent. and 20 per cent. respectively so that this mosquito is a vector elsewhere than in the immediate vicinity of Tambunan Plain. On the coast of North Borneo and in the interior the same vector was suggested by finding that malaria was intense in jungle-covered areas, and that it was slight or absent in cleared areas. Here also, *A. leucosphyrus* was the only mosquito found with a preference for human blood, while *A. maculatus* had a preference for animal blood.

On the east coast the same thing was found. Although not actually incriminated, the observation of local medical men suggested the importance of *A. leucosphyrus* and the danger of jungle (CARLIER, 1941 etc.).

As a parallel to these observations a study of the literature yielded a number of important facts which it is planned to publish in full later. It

was found that, in spite of its elusive habits, *A leucosphyrus* had been recorded in more localities in Borneo than any other mosquito, being referred to by more than a score of observers, and found in every locality so far examined. It appeared to be distributed throughout the whole of Borneo, wherever there was jungle shade. Again it was found to have been suspected as a vector of malaria on epidemiological grounds in at least five places in Borneo, and actually incriminated, as already stated, in one place in Dutch Borneo, and in an island off the south-east of Dutch Borneo, as well as in the three places examined during the work described in North Borneo.

This species appears to be distributed throughout the jungle which is almost universal in Borneo, and has been either suspected or incriminated in the north, east, south west, and in the very centre of the island. Further, an analysis of the available figures shows that *A leucosphyrus* has exhibited the highest index of natural infection of any anopheles so far studied by dissection in Borneo, and to have been consistently infected wherever it has been so studied.

Thus, in spite of its elusiveness, and of its innocent reputation in Malaya and elsewhere, *A leucosphyrus* has proved to be the most widely distributed, the most frequently suspected, the most frequently incriminated, and the most highly infected mosquito so far studied in Borneo, and therefore to be probably the most dangerous insect throughout the island.

In contrast to this, 212 dissections of *A maculatus*—all that could be obtained throughout Borneo—have proved to be negative, and this, together with the indirect evidence of its preference for animal blood, its absence from many malarious areas, and the absence of malaria from many areas where it breeds freely, supports the belief that this mosquito, for long regarded as the chief vector of malaria in Borneo, is actually harmless throughout the island.

*The Importance of Varieties*—During this work superficial observations suggested the existence of at least two types of *A leucosphyrus*, differing in their habits, and, therefore, probably in their relation to malaria and it would appear of importance to make a further study of this.

#### THE IMPORTANCE TO OTHER COUNTRIES OF THESE FINDINGS

The fact that *A leucosphyrus* was the only vector of malaria in Tambunan, and that this was such an elusive insect, made it difficult to find any answer to the question of malaria transmission there. What would have been the result of the work had another vector, for example, *A minimus*, been present in the locality, is quite certain. There is not a shadow of doubt that *A minimus* would have been incriminated, and *A leucosphyrus* would have been missed.

To what extent, therefore, is this occurring elsewhere? To what extent is *A leucosphyrus* carrying malaria where *A minimus*, or *A maculatus*, or

some other mosquito, is bearing all the blame, and where control measures are consequently inadequate or inappropriate?

A search of the literature shows not only that *A. leucosphyrus* has been incriminated in other places, and its infection generally regarded as some thing of a curiosity but that wherever it has been dissected in adequate numbers it has always been found infected.

*A. leucosphyrus* is a widespread mosquito, being distributed almost right across Asia. It is often so difficult to find that it is reasonable to suppose it to be more widespread than the records show. To what extent it is of importance throughout the East, and to what extent its importance has been overlooked, remains to be determined by further work. Ceylon, for example claims that it is a harmless mosquito, but the evidence for this rests upon a record of only four negative dissections over the course of many years. Malaya claims it to be a harmless mosquito but only one dissection, by HOOCHIN (1934) appears to have been recorded. *A. leucosphyrus* is considered to be a harmless insect in India, but no dissections can be traced, with the exception of a series by CLARK and CHoudhury (1941) in Assam where it was proved to be positive.

It is suggested that if dissections are more widely carried out—not always an easy matter on account of the difficulty of obtaining material even where it abounds—it may prove to be a dangerous vector in areas where it has never been suspected and it may be found that the occasional incriminations of the insect throughout Asia are not such curiosities as has been supposed. More dissections are necessary throughout its range, and this may demand a new technique for the capture of such an elusive insect.

#### MALARIA CONTROL.

The aim of malaria research is practical malaria control, and these discoveries are of no value unless applied to an effective campaign against the disease. With the incrimination of *A. leucosphyrus* as a vector in Tambunan, therefore it remained to be discovered what methods of control were most likely to be effective there, and to do this two things were required. It needed, firstly to be proved whether *A. leucosphyrus* control actually resulted in malaria control, and, secondly it needed to be shown what were the most effective and economical method of permanently accomplishing this.

Malarial Control by *A. leucosphyrus* Control.—Firstly in order to prove whether *A. leucosphyrus* control results in malaria control, a locality was selected with a spleen rate of 100 per cent. and where the spleen rate had been maintained at this figure for at least 5 years. It lay in a heavily jungled valley associated with about fifteen streams and over 150 *A. leucosphyrus* breeding places. This represented probably the most difficult area to control in the district, and was selected partly for this reason—in order to

make a convincing experiment—and partly because of the consistently high spleen rate, so that any reduction might not be attributed to chance

The breeding places were carefully defined and mapped, and then dusted weekly, by hand, with paris green, avoiding the breeding places of other species. It was hoped that this, by temporarily abolishing the breeding would be reflected in a reduction of malaria in the villages

For some weeks it was fairly effective, in that breeding was diminished, although not abolished. After some time, however, heavy rains drenched the jungle, washing away the paris green, and it was soon clear that breeding was no longer controlled. Thus, this experiment failed. It could not be expected to influence the degree of malaria in such a short time, and it proved that dusting with paris green is likely to be unsuitable as a means of malaria control where *A. leucosphyrus* is a vector

**Practical *A. leucosphyrus* Control**—Concurrently with this experiment in malaria control, parallel experiments were also being carried out to determine the most effective economical methods of *A. leucosphyrus* control

Throughout the greater part of Borneo, especially in the intensely malarious areas, anti malaria measures on conventional lines are out of the question for economic reasons. Oiling, dusting with paris green, drainage and drug therapy, for example, are quite impracticable in vast isolated areas inhabited only by a few thousand primitive people living in the utmost poverty, even if these measures are effective. The only hope for malaria control is such a land lies in the discovery of some simple method of controlling the one dangerous species of mosquito, a method which is cheap, permanent and practicable under existing conditions, and which if possible will go hand in hand with some agricultural or other activity of the people

The most hopeful way of accomplishing this appeared to lie in the discovery of some means of naturalistic control

**Naturalistic Control of *A. leucosphyrus***—It was noted that *A. leucosphyrus* in Tambunan always bred in clear spring water, with an acid reaction, pH 6.0 to 6.4, in the presence of dead leaves, in seepages, under dense jungle shade, and was never found in any other situation. This suggested that breeding might be controlled by changing any of these conditions, by admitting sunlight, by abolishing the dead leaves, by changing the reaction of the water or by polluting it, and it seemed obvious that all these might be accomplished by the simple clearance of jungle for a short distance around the breeding place, and cultivating it or admitting cattle, and an experiment was undertaken to investigate this

Three apparently identical seepages were selected, each breeding *A. leucosphyrus* consistently. The first of these was cleared, and the vegetation was left lying on the ground, the second was cleared and then burned and the third was left alone as a control

Disappointingly the first seepage continued to breed without interruption, and was still breeding 3 months later although some other species had also become established. The second seepage cleared some time later and burned immediately ceased to breed *A. leucosphyrus* and began to breed other species. The third seepage continued to breed without interruption, proving that the results in the controlled seepages were not due for example to weather conditions.

Thus it was suggested that the most effective method of controlling *A. leucosphyrus* might be the clearing and burning of jungle for a very short distance around the dangerous breeding places and with the failure of the paris green as a means of experimental control, it was decided to apply this method of clearing to the attempted control of malaria.

**Malaria Control by Naturalistic *A. leucosphyrus* Control.**—The same locality was selected as before, and 3 months were spent in attempting to clear all the seepages within half a mile of the malarious villages, and in making observations on the most effective ways of undertaking this. It was found that complete clearing and burning, for quite a short distance around dangerous seepages, generally for about 30 feet resulted in every case in the immediate disappearance of *A. leucosphyrus* breeding although this breeding tended to return with the growing up again of jungle.

Even better than this however it was found that only partial clearing for quite a short radius, with the admission of cattle to graze, resulted also in the immediate control of *A. leucosphyrus*—a control which was accelerated by the pollution of the water by the cattle and since the cattle grazed down the young shoots, the control was apparently permanent, transforming the previously inaccessible jungle-covered soil into turf.

It was shown that these clearings, being moist sheltered, with good soil, were ideal situations for cultivation, and even—although previously deadly—for housing sites, and that the creation of grassy lanes for grazing and access to the jungle, following the course of stream was a simple means of control, and a benefit to the natives.

Unfortunately this work was interrupted, when only half completed, by the occupation of the country by the Japanese troops. It was not possible to expect any reduction of the malaria with such widespread breeding still in progress. Thus the experiment failed as a demonstration of actual malaria control. It succeeded however in adequately demonstrating the success of the method as a means of immediate effective and apparently permanent *A. leucosphyrus* control.

**Other Evidence of Malaria Control by this Method.**—Although this experiment failed, owing to its non-completion, to control malaria there is a

considerable weight of evidence throughout Borneo that jungle clearance in this island, unlike Malaya and many other countries, results in malaria control

For example, all towns, being cleared of jungle, appear to be healthy in strong contrast to their highly malarious jungle-covered environs which provide the cases of malaria. Cultivated areas, like Tambunan Plain with its paddy fields, are healthy, although they may be malarious if close to jungle. Sunsurun, in the hills of Tambunan, had a spleen rate of 80 per cent in 1930 when still under jungle, which was reduced to 25 per cent in 1940, however, with the extension of jungle clearance. On the coast, the northern Chinese Settlement, Jesselton, provided a great many cases of malaria and many deaths when it was first established 20 years previously, but in 1939, with the abolition of jungle and its replacement with rubber, it had no malaria and a negligible spleen rate of 0.5 per cent. It was stated by WEBSTER (1941) that Miri, although previously very malarious, became quite healthy when extensively cleared of jungle about 1924, with only sporadic cases since, until 1940. There are other evidences of the disappearance of malaria with jungle clearance in Borneo, and no evidence has yet been found to the contrary.

Thus it would appear that in Borneo jungle clearance results in malaria control.

*The Importance of Jungle Clearance*—In most other countries, malaria control is carried out by measures which cost money, and which in themselves contribute nothing further to the prosperity of the land. Whether such measures, for example for the control of *A. maculatus*, could have been applied to such a primitive rural population as is found in Tambunan, and with the small financial resources available is however, very doubtful and before the discovery of *A. leucosphyrus* as the vector, the possibility of effective control seemed very remote indeed.

With the discovery of *A. leucosphyrus* as the vector in Tambunan however, control of malaria presents itself not only as a possibility, but as an activity which is cheap, easy, effective and permanent, able to be carried out by the natives themselves, if directed, in the course of their daily work, and above all, an activity in keeping with the agricultural and economic development of the country. Thus the simple methods described, of localized jungle clearance, should result in benefit not only to the health, but to the wealth, of the people of Borneo.

#### SUMMARY

*A. maculatus* has for long been regarded as the chief vector of malaria in Borneo. As a result of the work described, however, this mosquito has been shown to have little and probably no importance as a vector there.

*A. leucosphyrus* on the other hand, generally regarded as a more or less harmless insect, has been shown to be the most dangerous vector in North Borneo and on the existing evidence, probably throughout the greater part of the island.

This is important because measures which control the one may encourage the other. Thus measures directed against *A. maculatus* where *A. leucosphyrus* is the vector may not only be ineffective, but may increase the disease they are intended to eliminate.

*A. leucosphyrus* was found to be a very elusive insect, avoiding discovery and incrimination for nearly 2 years until demonstrated by less conventional methods.

This mosquito eluded search as a larva because it bred under dense jungle which often required to be cleared for its exposure, and because it remained submerged for abnormally long. It eluded search as an adult because it fed in the early hours of morning when its victims were asleep, and because it did not rest in the houses.

It is considered that had some more easily discovered vector been present, *A. leucosphyrus* would certainly have been missed. It is suggested that, since the mosquito is so elusive, it may be of wider distribution and greater virulence and importance in other countries than has hitherto been supposed. It is pointed out that, in spite of its innocent reputation in many countries, wherever it has been examined in adequate numbers it has always proved to be a vector.

Experimental measures for the control of *A. leucosphyrus* showed that this mosquito may be easily and permanently abolished and by measures which are in keeping with agricultural and industrial development of the land.

It is hoped to publish a full account of this work as a volume later.

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Work carried out by the British and Americans during the Burma Campaign, and shortly to be published, confirms the belief expressed in this paper that the mosquito incriminated by unorthodox technique in Borneo, although previously unconsidered, may prove to be of much wider importance than has hitherto been realized.—ED.

## ACTION OF GAMMEXANE ON ARTHROPODS OF MEDICAL AND VETERINARY IMPORTANCE

BY

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"Gammexane" is the name given to the gamma isomer of benzene hexachloride (1 2 3 4 5 6 Hexachlorocyclohexane). Unfortunately, in the past the name gammexane has been used indiscriminately for the gamma isomer and for the crude mixture of isomers which was formerly known as "666". In this and future papers gammexane refers to the gamma ( $\gamma$ ) isomer and references to other authors' work are interpreted in terms of the gamma isomer, so far as that is possible. The discovery, preparation and early development of the  $\gamma$  isomer as an insecticide has been described by SLADE (1945) in the Hurter Memorial Lecture. In the present paper results so far known or recorded are brought together to show the gaps which exist in the information available. Except where otherwise indicated, the results reported were obtained in these laboratories and are in the course of preparation for publication. For convenience, the main orders and families of insects, ticks and mites are dealt with briefly, giving comparable information for DDT where known.

The experimental preparation used was (except where otherwise stated) a dilution in water prepared from a 5 per cent solution of gammexane in a mixture of sulphonated castor oil and an organic solvent in proportions to give an easily pourable, miscible oil. Where comparison with DDT was made a preparation of technical DDT in the same base was used.



## INSECTA (HEXAPODA).

## DIPTERA.

## (a) ORTHORHAPHA.

So far as it has been tested gammexane shows a very high degree of activity against the larvae as well as the adults of this suborder.

**CHIRONOMIDAE.** *In vitro* testing against the aquatic larvae of *Culiseta inornata* with observations made after 24 hours of continuous contact showed a dilution of 1 in 5,000,000 of gammexane to be effective.

**SIMULIIDAE.** *In vitro* testing against the larvae of *Simulium* spp. with exposure to the insecticide for 1 hour followed by a change of water showed complete mortality (after 24 hours) with gammexane at 1 in 8,000,000 and almost complete mortality with DDT at 1 in 4,000,000. 30 per cent. survived DDT at 1 in 6,000,000 and 50 per cent. at 1 in 8,000,000. Against the adults of these families and both adults and larvae of the *Psychodidae* (*Phlebotomus*) no data are available.

**CULICIDAE.** The toxicity of benzene hexachloride to both the larvae and the adults of *Culicidae* is of a similar order to that of DDT. A paper on this aspect is at present in the press (RIBBANT) and further field trials are in progress abroad.

## (b) CYCLORHAPHA (MUSCIDAE CALYPTERATAE)

From the evidence available to date gammexane shows a high degree of activity against the adults of this suborder but rather less activity and in some cases (*Gastrophilus* and *Hypoderma*) considerably less activity against the larvae as compared with the orthorrhaphous diptera.

**Musca.** Against the house fly good residual activity is obtained by treating strategic points—landing places and roosts such as electric bulbs and shades and tops and bottoms of window panes, etc. With 0.35 per cent. solution in kerosene, the effect lasts for at least 4 weeks. On the larvae good results have been claimed by use of a dust containing 0.5 per cent. gammexane when applied to refuse heaps and other breeding places.

**Stomoxys.** Against the stable fly residual activity lasting up to 3 weeks was obtained by a deposit on stable walls approximating 40 mg. per square foot. No data are available concerning the larvae.

## Hippoboscinae.

**Metophaeus ovinus** (sheep ked). Residual activity persists for more than 3 weeks in long-wooled sheep treated with a 0.004 per cent (1 in 25,000) dilution. Keds appear to have been eradicated from one small flock treated with this dilution. DDT was used successfully at 0.1 per cent. by CORBETT and SMITH (1945) and 0.5 per cent. by HEATH (1945).

## Calliphorinae

Against the sheep blowfly (*Lucilia* spp), HARBOUR and WATT (1945) obtained good protection for up to 6 weeks with gammexane used as a spray at 0.5 per cent. All strikes occurring in treated sheep were around the tail on dirty wool.

## Gastrophilinae

Larvae of *Gastrophilus intestinalis* were more or less unaffected after 24 hours' exposure to gammexane at 1 in 2,500.

## Hypodermatinae

Larvae exposed to concentrations of up to 1 in 100 of gammexane were not all killed—solution applied to the backs of infested cattle in the manner in general use with derris.

## SIPHONAPTERA

*Hypoderma* larvae. No exact data available but good results have been reported with the use of 0.5 per cent gammexane applied as a dust to the breeding places.

Adults of *Ctenocephalus* on dogs are killed by bathing in dilutions of 1 in 15,000 (0.0075 per cent).

## ANOPLURA (Siphunculata and Mallophaga)

Dilutions of from 1 in 80,000 to 100,000 (0.00125 to 0.001 per cent) are lethal to sucking and biting lice (*Haematopinus asini*, *Bovicola bovis*) as compared with DDT at 1 in 10,000 (*Bovicola*).

For complete control with a single application the following concentrations appear effective against different species.

*Bovicola*. Usually at 1 in 30,000 and higher concentrations (DDT incomplete at 1 in 8,000 once, complete at 1 in 5,000).

*Haematopinus eurysternus*. Higher concentrations appear necessary, possibly because these lice are found in places where hair is sometimes short or sparse. In one case 1 in 10,000 failed to give complete control on rather bare skin of the udder, whilst in another 1 in 30,000 appeared to give complete control over a neck infection.

*H. asini*. Complete control seems to have been attained in one case by 1 in 100,000 (single application) whereas in another the same dilution was effective for 7 weeks only.

*H. suis*. Single application of a dilution of 1 in 20,000 (0.005 per cent) gammexane in an oil spirit lotion freed pigs from *H. suis*—no lice being seen up to 27 days (last observation). DDT was used by KEMPER and ROBERT (1946) at 0.75 per cent to free pigs from the same louse also by single application.



## ARACHNIDA

## ACARINA

## MESOSTIGMATA

ASTIGMATA  
(Sarcoptoidea)

Genera  
*Notoedres*—1/10,000  
 single application  
*Chorioptes* } Promising  
*Sarcoptes* } results  
*Psoroptes*—No data

HETEROSTIGMATA  
(Tarsonemidae)

*Acarapis*

PROSTIGMATA  
(Trombididae)VERMIFORMULA  
(Demodicidae)ARGASIDAE  
(Soft ticks)

Genera *Argas*  
*Ornithodoros*  
 150 mg /sq foot twice  
 at 3 weeks eradicates  
 —FOCKING (1946)

IXODIDAE  
(Hard ticks)

Genera *Boophilus* (0.008%  
 inhibits laying—  
 WHITNALL)  
*Rhipicephalus*  
*Ixodes*, etc.

## GAMASIDAE

*Dermanyssus* 1/2,500  
 spray—almost complete  
 eradication

## CUTITERIBRINAE

*Dermatobia*

## GASTROPHILINAE

*Gastrophilus*  
 (Horse bots)

Larvae—relatively  
 ineffective

## HYPODERMATINAE

*Hypoderma*  
 (Warbles)

Larvae—incomplete  
 effect at 1 in 100  
 Adults—no data

## OESTRINAE

*Oestrus*  
 (Nasal bots)

No data

*Trichodectes canis* and *Linognathus setosus* were eradicated from dogs bathed once in dilutions of 1 in 20 000 and sometimes even higher dilutions. DDT at 1 in 10 000 failed to eradicate *T. canis*.

*Columbicola columbae* in the pigeon was killed by 0.01 per cent. dip.

### HEMIPTERA (Bugs).

Against reduviid bugs trials are in progress in South America, but against *C. mex lectularius* BARNES (1945) found that there was 100 per cent. kill by direct spray of 0.0004 mg per sq. cm. compared with 91 per cent. kill with DDT at 0.004 mg per sq. cm. Under the conditions of the experiment, the residual activity of DDT persisted longer than that of gammexane. BURVINE (1946) has shown that the median lethal concentration of gammexane to *Cimex lectularius* is 0.015 per cent. compared with 0.5 per cent. for DDT when used as a dust. Trials in infested property show that practical control of bed bugs can be obtained and re-infestation almost completely prevented by the use of gammexane as a 0.35 per cent. solution.

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#### Argasidae (soft ticks)

Only rather general information is available that it is active against *Argas persicus* (fowl tick) but against *Ornithodoros moubata* (human tick, Africa) HOCKING (1946) found that spraying the soil in barrack huts twice at an interval of 3 weeks eradicated the tick, whilst DDT reduced the tick population but was not so effective at the same dilution. HOCKING mentions the dilution of

666 as being 1,250 mg per sq. foot which is equivalent to about 150 mg of the  $\gamma$  isomer per square foot. Presumably DDT was used at 1,250 mg per square foot or eight times the concentration of gammexane.

#### Ixodidae (hard ticks).

Little exact information is available, but WHITNALL (unpublished information) reports that in laboratory experiments 0.008 per cent. gammexane in emulsion was completely effective in inhibiting the egg laying of *Boophilus decoloratus*. Similar activity was shown by 0.32 per cent.  $As_2O_3$  (as sodium arsenite in water) and 4 per cent. DDT emulsion. AULT (1946) working with *Boophilus australis* in South America, found that either a 1:1700 concentration of the  $\gamma$  isomer alone or 1:8000 concentration in addition to a plain arsenical dip with 0.2 per cent.  $As_2O_3$  gives complete control of the tick.

#### ASTIGMATA (SARCOPTOIDEA)

TAYLOR (1945) was the first to demonstrate the acaricidal activity of gammexane (using *Notoedres muris*) and its superiority in this respect to DDT.

J S STEWARD

and other commonly used acaricides TAYLOR used 0.1 per cent gammexane in liquid paraffin and used two applications. It has since been discovered in these laboratories that against *Notoedres muris* it is effective by single immersion for half a minute in dilutions up to 0.01 per cent.

Single spray treatment of choriopic leg mange in horses and neck and rump mange in cattle has resulted in destruction of most of the acar and great clinical improvement with dilutions up to 1 in 40,000 DDT at 1 in 10,000 did not kill the majority of *Chorioptes bovis*.

Exact data on *Sarcoptes* spp. are not available though some favourable results have been obtained with this deep living species. Clinical recovery of affected pigs has followed single application of gammexane at 1 in 2,000. Following a report by ELMES (1945) that DDT at 5 per cent in acetone was effective against notoedric ear mange in rabbits, TAYLOR reported fatal toxic symptoms with this concentration and subsequently KIRBY (1945) stated that 5 per cent "666" (= 0.6 per cent  $\gamma$ ) was innocuous when applied eighteen times in 3 weeks to ears, paws, nose and tail of rats.

#### Gamasidae

*Dermanyssus* (red mite) in poultry houses has been strongly reduced by single spraying with gammexane at 1 in 5,000 but not completely eliminated. Almost complete elimination has followed the use of 1 in 2,500.

POVAR (1946) found 10 per cent DDT incompletely effective against *Liponyssus sylvarum* (northern feather mite) and less effective than nicotine. He quotes a U.S.D.A. report that DDT was ineffective for the control of the poultry mite (*Dermanyssus gallinae*).

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# DISTRIBUTION OF ADULT MOSQUITOES BY RESIDUAL DDT METHODS

BY

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I am indebted to the ACTING DIRECTOR OF MEDICAL SERVICES, Gold Coast, for permission to publish and to both Dr H. P. FOWLER, former Senior Health Officer, Gold Coast, and Wing Commander C. G. HAROLD, Royal Air Force, for generously placing the resources of the Fakoradi Malaria Control Scheme at my disposal. Acknowledgement is due also to the Scheme's subordinate personnel for loyal and efficient service and to the *Insecticides News-Letter* service of the Ministry of Production for providing my only available source of appropriate scientific information.

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## I.—INTRODUCTION

The spray killing of mosquitoes in houses forms a major feature of routine anti-malaria measures undertaken in the Takoradi area of the Gold Coast. Details of the organization employed and the results obtained from the repeated use of various insecticides prior to 1944 have been described by EDDY (1944). As soon as supplies of DDT became available trials were undertaken with a view to ascertaining whether the repetitive spraying procedures used previously could, with advantage, be replaced by residual DDT spraying methods.

## II.—PRELIMINARY TRIALS.

### (1) *Objects*

In the first instance trials of a preliminary nature only were attempted since the prevailing dry season conditions had left relatively few mosquitoes available by which to measure results. The main purpose of these trials was to afford experience as to the best practical spraying procedures to be adopted under local conditions. They were designed also to provide tentative answers to the following questions —

(i) Whether a 1 per cent. solution of DDT in kerosene would demonstrate residual insecticidal effects against house-infesting mosquitoes under local conditions.

(ii) The period of time over which effects achieved by a specific dosage of DDT might be expected to persist without substantial loss of efficiency

(iii) The extent to which a marked variation in the dosage of DDT per square foot of sprayed surface would modify the period of efficient persistence.

(iv) The relative merits as a residual DDT spraying apparatus of two standard types of sprayer

### (2) *Location.*

The site chosen for the first three trials was a housing estate (Adiembra) which offered two important advantages firstly it was served already by experienced spray personnel familiar with the individual compounds and known to their tenants secondly the contained rooms were identical in every respect and facilitated therefore the use of alternate compounds as control units. A disadvantage common to all the local sites at the time, was that the routine employment of comprehensive larvicidal control measures supplemented the dry season conditions already mentioned in limiting the numbers of house-infesting mosquitoes available.

### (3) *Spraying Procedure*

Two spraying teams, each comprising one recorder, one semi-literate headman and nine labourers, were employed. Initially, the ceiling and/or walls of each room in alternate compounds were given a residual spraying with a 1 per cent solution of DDT in kerosene. This solution was prepared by diluting quantities of a 20 per cent DDT liquid concentrate with ordinary commercial kerosene in the proportions of nineteen parts of kerosene to one part of concentrate. Thereafter, at either once-weekly or twice-weekly intervals, both the compounds originally treated with DDT and the alternate compounds used as controls were uniformly check-sprayed with a pyrethrum-kerosene solution. The latter was prepared by adding half a pound of dry pyrethrum to each gallon of commercial kerosene and effecting a double extraction over a period of 48 hours.

The procedure adopted for these check-spraying rounds was that a team of five labourers under the headman, having first searched for and collected any dead mosquitoes in the rooms, then sprayed two rooms at a time. Two sprayers worked inside and one outside each room simultaneously, the last-mentioned being employed to create a barrier zone of insecticide over the previously closed window and door apertures. After a 15-minute interval the two sprayed rooms were entered by the remaining four labourers. Two labourers swept each room under the supervision of the recorder, all dead and stupefied mosquitoes being collected and counted. In conformance with routine experience, full co-operation was obtained from the general public but complete spraying of every compound proved impracticable owing to a number of rooms remaining locked in the absence at work of their respective tenants.

### (4) *Details of Trial I*

The zone selected for this particular trial contained a total of 930 rooms arranged in groups of approximately sixty rooms each and housed an average total of 2,620 persons. Each room measured 12 ft by 10 ft by 8 ft 9 in high giving a combined ceiling and wall area of 505 sq ft. All rooms contained a ceiling and were equipped with two louvred windows, each measuring 3 ft 8 in by 2 ft 8 in, and also one well-fitting door. The internal wall surfaces had been plastered to a smooth finish and limewashed.

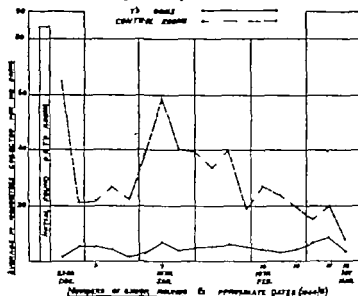
A 1 per cent DDT solution was applied with "Four Oaks" knapsack sprayers of external-pump type fitted with a single nozzle having a  $3/32$  in opening. Their  $3\frac{1}{4}$  gallon capacity containers were only partially filled to avoid spilling and care was taken generally to minimize the risk of possible toxic effects resulting from excessive skin and clothing contact with the insecticide. The dosage employed aimed at just completely moistening the surface of both walls and ceiling. An average of 13.8 fluid ounces of insecticide was used per 100 sq ft of surface, i.e., 69.5 fluid ounces per room, giving a calculated

deposit of 38.5 mg DDT per sq. ft. For the check rounds an average per room of 1.8 fluid ounces of pyrethrum-kerosene was sprayed from continuous flat gun types of domestic hand pump.

The initial results obtained are given in Fig. 1 and Table I. They demonstrate a prolonged relative absence of mosquitoes in the DDT'd rooms. It will be noted that, whilst the actual numbers of mosquitoes collected diminished steadily presumably because of the elimination of natural breeding places as the dry season lengthened, mosquitoes were never wholly absent and the kills

FIG. 1. TAKOMADI AREA—DDT TRIAL I

Graph of mosquitoes collected.



in the control rooms remained throughout proportionately greater than the kills in the DDT'd rooms.

In Fig. 2 is shown a DDT index graph recording, as a decimal fraction for each check round, the actual proportion of DDT'd room mosquito collections to control room collections. It indicates a gradual reduction of the high initial disparity between the mosquito populations in the DDT'd and control room groups but demonstrates that a substantial degree of DDT efficiency persisted for at least 2 months from the original date of spraying.

#### (5) Details of Trial II

For Trial II, a zone was chosen comprising 108 rooms arranged in identical blocks of four and housing an average total of 298 persons. Each room measured 14 ft. by 12 ft. by 10 ft. 3 in. thus giving a total wall area of 533 sq. ft. All

TAKORADI AREA MOSQUITOES COLLECTED IN DDT TRIAL NO 1

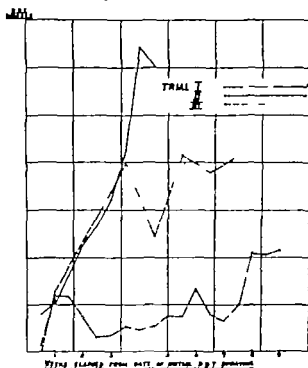
S. G. EDDEY

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TAKORADI AREA MOSQUITOES COLLECTED IN DDT TRIAL NO 1

Spraying period 1944/45	Number of check round	DDT d rooms			Control rooms			DDT indexes	
		Number of rooms sprayed and swept	Mosquitoes collected after spraying		Number of rooms sprayed and swept	Mosquitoes collected after spraying			
			Actual numbers	Average numbers per 100 rooms		Actual numbers	Average numbers per 100 rooms		
December 20-22	Initial DDT spraying 1	368	313	85.1	—	—	—	—	
23-28 Dec-Jan 28-1	2	340	6	1.8	313	203	64.9	0.03	
January 2-5	3	365	19	5.2	336	73	21.7	0.24	
5-9	4	350	18	5.1	347	77	22.2	0.23	
10-12	5	372	15	4.0	363	98	27.0	0.15	
13-17	6	355	5	1.4	366	81	22.8	0.06	
17-19	7	351	10	2.9	341	132	38.7	0.07	
20-24	8	346	22	6.4	340	200	58.8	0.11	
24-27	9	356	14	3.9	355	145	40.9	0.10	
Jan-Feb 28-1	10	350	15	4.3	345	137	39.7	0.11	
February 1-5	11	336	17	5.0	339	116	34.2	0.15	
5-8	12	338	20	5.9	347	140	40.3	0.15	
8-12	13	338	17	5.0	338	64	18.9	0.27	
12-15	14	330	14	4.2	303	83	27.4	0.16	
15-19	15	313	10	3.2	342	84	24.6	0.13	
20-22	16	325	13	4.0	342	68	19.9	0.20	
24-27	17	316	20	6.3	306	55	15.0	0.42	
Feb-Mar 28-2	18	319	26	8.2	346	68	19.7	0.41	
		323	11	3.4	345	27	7.8	0.44	

FIG. 2. TANORADI AREA—DDT TRIALS I, II AND III.  
Graph of DDT index trends.



rooms contained a ceiling and were equipped with two louvered windows, each measuring 3 ft. 9 in. by 2 ft. 8 in. and also two well fitting doors.

A 1 per cent. DDT solution dispersed from Four Oaks knapsack sprayers as described for Trial I was again employed. Since however the principal object of this trial was to ascertain to what extent a substantially reduced dosage would diminish the period of efficient persistence the direct spraying of ceilings was omitted altogether and no attempt was made to moisten the entire wall surfaces. Instead, they were given a liberal scattering of fine droplets the greater number being concentrated on sections of wall either screened behind open doors or so remote from windows as to be especially likely to attract mosquitoes in search of a resting place. The only droplets deposited on the ceilings were those received indirectly from the process of spraying adjacent wall tops. An average of 4.2 fluid ounces of the insecticide were used per 100 sq. ft. of surface sprayed, i.e. 22.4 fluid ounces per room, giving a calculated deposit of 11.9 mg. DDT per sq. ft. The consumption of pyrethrum-kerosene for the check rounds averaged 2.5 fluid ounces per room.

The results obtained are shown in Table II and Fig. 3. They demonstrate

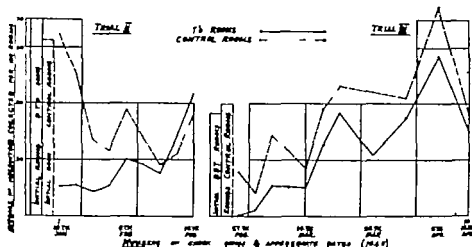
TABLE II  
TAKORADI AREA MOSQUITOES COLLECTED IN DDT TRIAL NO II

Spraying period 1945	Number of check round	DDT d rooms			Control rooms			DDT indexes
		Number of rooms sprayed and swept	Mosquitoes collected after spraying		Number of rooms sprayed and swept.	Mosquitoes collected after spraying		
			Actual numbers	Average numbers per 100 rooms		Actual numbers	Average numbers per 100 rooms	
January 16	Initial DDT spraying	50	35	70.0	46	29	63.0	—
19	1	50	5	10.0	42	27	64.3	0.16
21	2	48	5	10.4	44	22	50.0	0.21
27	3	46	4	8.7	44	12	27.3	0.32
31	4	48	5	10.4	48	11	22.9	0.45
February 5	5	50	10	20.0	48	18	37.5	0.53
8	6	44	8	18.2	46	13	28.3	0.64
12	7	46	7	15.2	50	9	18.0	0.85
15	8	46	13	28.3	50	11	22.0	1.28
19	9	46	20	43.5	50	18	36.0	1.21



FIG. 3. TAKEDOWN AREA—DDT TRIALS II AND III.

Graph of mosquitoes collected.



a relative absence of mosquitoes in the DDT'd rooms but for a much shorter period and with much less disparity than was the case for Trial I. It will be noted that in the low dosage employed DDT became relatively inefficient after the third check round, *i.e.*, a period of 11 days from the date of initial spraying. Complete parity with the control result was reached in a total of 28 days. Fig 2 enables the DDT index graph to be compared with that for Trial I, the respective rounds being plotted on equivalent time interval bases. The comparison emphasizes the rapid loss of efficiency and the much reduced period of persistence likely to ensue from using the smaller dosage of insecticide.

### (8) Details of Trial III

The object of Trial III was to ascertain whether more efficient results could be obtained by substituting the Four Oaks Maney sprayer for the knapsack sprayer. Accordingly Trial II was repeated using exactly the same rooms and dispersing the same dosage but from Maney type sprayers of 1 pint capacity and fitted with standard straight nozzles having a  $3/64$  in. opening.

The results obtained are shown in Table III and Fig 3. Much the same degree of disparity and period of persistence is demonstrated as for Trial II in the early stages. Later mosquito numbers collected showed considerable fluctuation—probably on account of the earlier dry season conditions having begun to give place to occasional rain periods by this time. As plotted together in Fig 2 the respective DDT index graphs confirm that using equal dosages of insecticide the two types of sprayer produce almost identical standards of efficiency up to near parity level.

TABLE III  
TAKORADI AREA MOSQUITOES COLLECTED IN DDT TRIAL NO III

Spraying period 1945	Number of check round	DDT'd rooms			Control rooms.			DDT indexes
		Number of rooms sprayed and swept.	Mosquitoes collected after spraying		Number of rooms sprayed and swept	Mosquitoes collected after spraying		
			Actual numbers	Average numbers per 100 rooms		Actual numbers	Average numbers per 100 rooms	
February 23	Initial DDT spraying	52	19	38.6	48	19	39.6	—
27	1	50	—	0.0	52	8	15.4	0.00
March								
2	2	49	1	2.0	50	4	8.0	0.26
7	3	48	5	10.4	48	14	29.2	0.36
14	4	51	5	9.8	52	9	17.3	0.57
17	5	47	12	25.5	50	19	38.0	0.07
22	6	40	17	37.0	52	24	46.2	0.80
28	7	51	11	21.6	50	22	44.0	0.49
April								
5	8	40	17	34.7	50	21	42.0	0.83
11	9	49	28	57.2	48	36	75.0	0.76
18	10	47	14	29.8	50	18	36.0	0.83

From the practical standpoint, however the Mancy sprayer required a much longer time than the knapsack to spray a given dosage and is more fatiguing to use. Its smaller capacity too, introduces more frequent filling delays. In the absence of any compensatory enhancement of efficiency these disadvantages make the Mancy apparatus much less suitable than the knapsack sprayer for large-scale work.

### III—MAIN TRIALS Nos. IV AND V

#### (1) TRIAL IV (APOWA)

(i) *Area Sprayed.* The foregoing preliminary trials had been undertaken in an urbanized portion of the Takoradi area. Here, mosquito numbers were restricted by the routine application of larvicidal control measures. Trial IV was introduced as an attempt to test DDT under backward semi rural conditions. It was conducted in a village (Apowa) sited a distance of 4 miles from the nearest limit of the Takoradi malaria control area. Apowa a total of 925 rooms were found to accommodate an average of 1911 persons. During the dry season uncontrolled mosquito breeding was observed regularly in a number of water holes on the village outskirts. As the wet season developed these collections of stagnant water became the focal points of undrained marshy areas. The two main streets of this roughly circular village are placed at right angles to one another and serve to divide it into four approximately equal quadrants. Opposite pairs of quadrants were used as control and DDT'd sections respectively so as to ensure that the infestation rate in each would be fairly even.

(ii) *Spraying Procedure.* The technique followed was identical with that described using knapsack sprayers as in Trial II excepting that the dosage employed was intermediate to those for Trials I and II being 7.05 ounces of 1 per cent. DDT solution per 100 sq. ft. of sprayed surface giving a calculated deposit of 19.75 mg. DDT per sq. ft. Check spray rounds were effected at regular weekly intervals and the main results obtained are shown in Table IV and Fig. 4

(iii) *Resulting Effects on Mosquito Infestation.* It will be observed that, despite its gradual increase over the experimental period, the DDT index factor had not quite attained to its initial level at the end of 9 months. Under the control conditions described as prevailing throughout the trial the DDT treatment would seem to have been solely responsible for the prolonged though steadily diminishing relative absence of mosquitoes in the DDT'd sections of the village.

As regards positive findings, Table IV records the presence of numbers of dead mosquitoes in the DDT'd rooms prior to each of the first ten check rounds this finding despite the daily room cleansing undertaken by the average occupant and in contradistinction to the complete absence of any dead mosquitoes in the control rooms prior to check spraying. It seems clear that

FIG 4 TAKORADI AREA (APOWA)—DDT TRIAL IV  
Graph of mosquitoes collected and DDT index trends

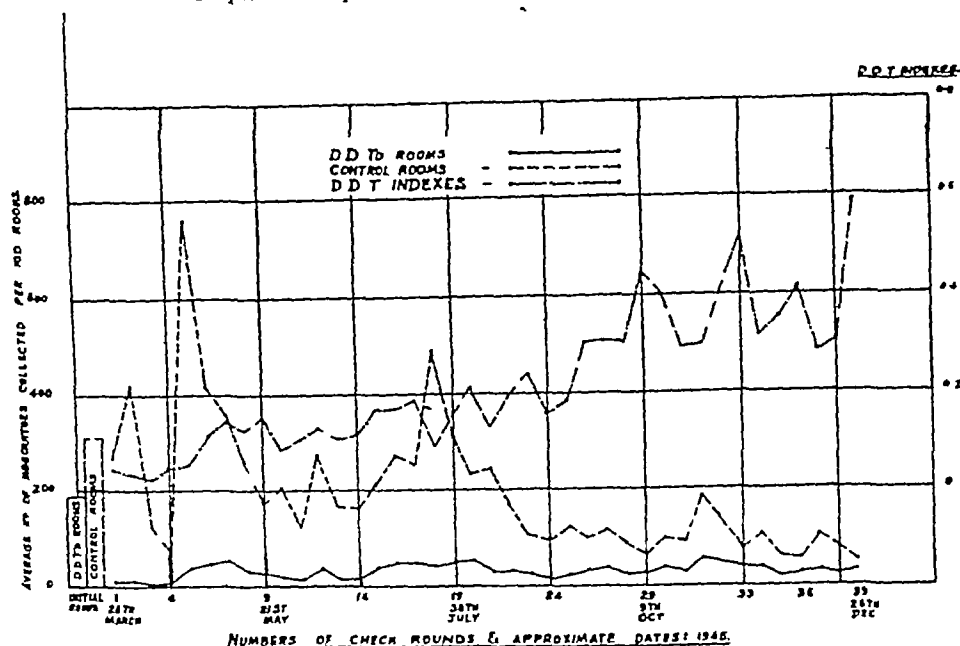
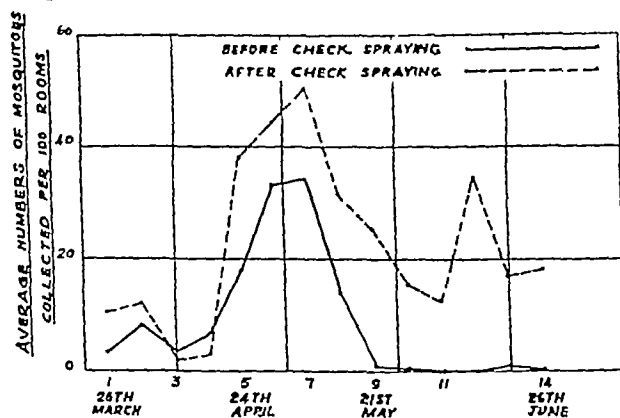


FIG 5 TAKORADI AREA (APOWA) DDT TRIAL IV  
Graph of mosquitoes collected in DDT'd rooms before and after check spraying



during the 10-week period involved sustained lethal effects were being obtained between check rounds in the DDT'd rooms though not in the control rooms Table V and Fig 5 indicate that, quantitatively, these pre-check spray collections reflected fairly closely the fluctuations in total mosquito prevalence as revealed by the collections made after check spraying

TABLE IV  
TANZANIA AREA (ARWA) MOSQUITOES COLLECTED IN DDT TRAIL, NO. IV

Spraying period 1945	Number of check rooms treated	DDT'd compounds				Control compounds				DDT Index
		Number of rooms sprayed and swept	Number of mosquitoes collected		Average per 100 rooms after spraying	Number of rooms sprayed and swept	Number of mosquitoes collected		Average per 100 rooms after spraying	
			Actual before spraying	Actual after spraying			Actual before spraying	Actual after spraying		
March										
18-21	lasted DDT spray	244	—	722	187.6	246	—	1186	203.0	0.61
28-31	1	246	12	38	16.7	249	—	925	264.1	0.04
April										
2-4	2	378	31	46	12.2	370	—	1,550	418.9	0.03
8-10	3	290	14	9	3.4	281	—	432	125.2	0.09
14-15	4	280	28	15	3.1	260	—	258	71.1	0.04
22-24	8	281	60	144	37.8	269	—	2,844	789.6	0.06
May										
1-2	6	342	127	171	44.8	292	—	1,634	418.8	0.11
7-9	7	381	121	183	50.7	296	—	1,277	254.7	0.14
14-15	8	375	42	118	21.8	292	—	1,014	253.7	0.13
21-22	9	381	4	97	25.5	292	—	678	172.8	0.15
28-29	10	386	2	60	16.0	289	—	791	202.2	0.08
June										
4-6	11	290	—	48	12.6	292	—	482	122.8	0.10
11-12	12	278	—	129	24.8	291	—	1,024	266.6	0.13
18-19	13	281	4	68	17.2	290	—	641	167.4	0.10
25-26	14	281	—	70	19.4	282	—	616	166.6	0.11

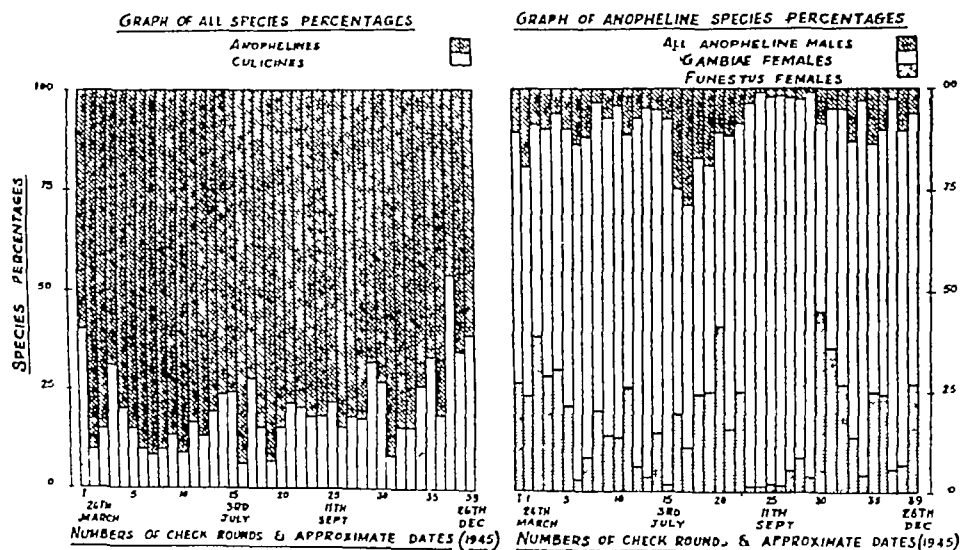


TABLE V  
YAKOMA AREA (ALOWA) DDT TRIAL NO. IV  
MOSQUITOES COLLECTED IN DDT'S ROOMS, BEFORE AND AFTER CREEK SPRAYING.

Spraying period 1945.	Number of check rounds.	Number of rooms sprayed and traps.	Mosquitoes collected before spraying.		Mosquitoes collected after spraying.	
			Actual numbers.	Average numbers per 100 rooms.	Actual numbers.	Average numbers per 100 rooms.
March						
18-21	Local DDT spraying 1	358	—	—	722	187.8
24-27		386	12	3-4	34	10.7
April						
2-4	3	376	31	8.2	45	12.2
4-10	2	280	14	2.7	9	2.4
14-18	4	280	74	6-8	12	3.1
23-26	5	341	69	12-1	144	37.8
May						
1-2	6	382	127	32.2	171	44.8
7-9	7	331	131	34-4	183	50.7
14-16	8	278	52	12.9	118	31.5
21-23	9	381	4	1.1	97	23.8
28-29	10	282	2	0.8	60	18.6
June						
4-6	11	330	—	—	48	12.8
11-12	12	378	—	—	129	31.4
18-19	13	282	4	1.2	66	17.2
25-26	14	381	—	—	70	18.4

(iv) *Entomology* Fig 6 and Table VI record both the anopheline, culicine proportions and an anopheline species differentiation of all mosquitoes collected throughout the trial period. The predominance of *Anopheles gambiae*, the principal local vector of malaria, will be noted. All other anophelines identified were recorded as of a second vector species, *A. funestus*, excepting for the appearance of *A. obscurus* in the collections on very rare occasions. Culicines identified from time to time by Staff Sergt F R P LEMMON, R A M C, who ably supervised also the trained African workers responsible for routine identification of the anopheline collections, were *Aedes simpsoni*, *Aed domesticus*, *Aed longipalpis*, *Uranotaenia annulata*, *Culex thalassius*, *C duttoni*, *C nebulosus*, *C tigripes* and *Aed aegypti*, the four last named in substantial numbers.

FIG 6 TAKORADI AREA (APOWA)—DDT TRIAL IV  
Identification of mosquitoes collected



## (2) TRIAL V (TAKORADI TOWNSHIP)

(i) *Area Sprayed* From the results of the preceding trials it was concluded that DDT residual spraying would prove as efficacious in the Takoradi area as had been claimed for it elsewhere. Further, that a single application of the relatively economical 1 per cent solution in a dosage approximating to 20 mg DDT per sq ft. would suffice to give reasonably efficient lethal results over a period of 2 months or more. Accordingly, arrangements were made to discontinue the routine twice-weekly spraying with pyrethrum-kerosene solution of native dwellings in the Takoradi township and neighbouring village areas in favour of residual DDT treatment. In addition, DDT spraying was extended



TABLE VI.  
TAKOBASH AREA (APOWA): DOT TRIAL NO. IV  
IDENTIFICATION OF MOSQUITOES COLLECTED.

Spraying period 1945	Number of check round.	Anopheles percentage of total mosquitoes collected.	Differentiation of anopheles species.			
			Percentages of females.		Percentages of males.	
			<i>A. gambiae</i>	<i>A. funestus</i>	<i>A. gambiae</i>	<i>A. funestus</i>
March 19-1	Initial DDT spraying	68.28	81.73	27.23	8.66	8.29
24-27	1	90.46	86.79	24.16	14.22	4.84
April 3-4		85.41	83.83	26.89	6.33	2.65
9-10	2	68.63	86.43	29.14	7.66	3.37
14-18	4	80.67	83.99	30.89	4.73	1.29
23-24	8	88.30	68.31	31.38	7.78	2.98
May 1-2	6	80.08	82.61	2.78	12.74	0.82
7-8	7	91.80	78.60	8.47	9.66	2.47
14-18	8	90.60	77.63	19.06	1.34	1.93
21-22	9	94.60	78.61	14.16	3.18	4.08
28-29	10	91.28	82.73	12.43	3.02	0.82
June 4-5	11	84.00	62.80	28.69	8.04	3.37
11-12	12	86.78	86.46	6.34	8.76	1.44
18-19	13	81.00	91.67	3.39	3.09	1.86
25-26	14	78.25	80.00	14.43	8.24	0.23
July 3	15	76.08	91.12	1.32	6.21	1.39
9-11	16	82.78	86.01	19.30	16.62	8.28
16-17	17	72.00	89.72	11.11	25.00	4.17
23-24	18	88.18	88.94	24.65	13.49	3.82
July-Aug. 30-1	19	83.28	56.30	24.67	12.84	8.09
Aug. 7-8	20	83.80	47.93	41.83	7.66	92
13-14	21	78.78	73.02	18.83	6.35	4.78
20-21	22	79.28	66.66	24.92	8.09	2.82
27-28	23	82.28	93.13	1.22	3.24	0.30
Sept. 3-4	24	81.78	97.44	1.22	0.31	61
10-11	25	78.00	85.89	1.82	1.26	0.86
17-18	26	85.00	94.44	1.77	0.69	1.18
24-25	27	82.00	91.77	8.79	1.82	6.82
Oct. 1-2	28	82.78	88.22	8.76	1.41	1.21
8-9	29	68.61	93.78	3.39	0.85	0.00
15-16	30	73.78	66.78	44.75	4.78	3.78
22-23	31	92.28	88.85	26.22	2.97	2.18
29-30	32	84.78	69.14	29.64	2.66	2.30
Nov. L. 13	33	85.09	73.16	13.66	8.85	4.13
19-20	34	74.09	82.85	4.28	2.70	0.25
26-27	35	66.69	61.34	24.72	11.29	2.48
Dec. 3-4	36	81.81	86.27	23.81	7.14	2.78
11-11	37	46.78	91.45	8.83	1.0	1.09
17-18	38	83.42	82.71	6.59	9.47	0.83
24-27	39	81.65	63.92	27.38	8.79	3.61

to a limited number of villages and isolated African quarters sited within  $1\frac{1}{2}$  miles of the township and likely therefore to harbour mosquitoes capable of infiltrating on occasion into the controlled area

(ii) *Spraying Procedure* Knapsack sprayers and the technique described for Trial II were again employed but the 1 per cent DDT solution was prepared by dissolving 60 per cent strength DDT powder in ordinary commercial kerosene, 6  $\frac{2}{3}$  lb of the powder being added to each 40 gallons of kerosene. The mixing took place in drums of 44 gallon capacity. At first the dry powder was added to the kerosene direct, the mixing drum being placed in the sun for 2 whole days and its content subjected to agitation by periodic rolling of the drum over a distance of 20 yards. Later, it became the practice to melt the powder by heating it in a metal pan over a small wood fire immediately before adding it to the kerosene. Care was taken to remove all coarse suspended matter from the insecticide before using it in the sprayers. Passage through the wire gauze strainer placed beneath the filling cap of the Four Oaks container proved satisfactory for this purpose.

(iii) *Insecticide Dosage* Table VII records the spacing of the DDT spray rounds made in the centralized portion of Takoradi township during

TABLE VII  
DETAILS OF DDT SPRAY ROUNDS TAKORADI TOWNSHIP, 1945

Number of round	Date commenced	Date completed	Total rooms sprayed	Total insecticide used in gallons
1	March 28th (Interval	April 24th 1st-2nd round	3,002 11 $\frac{1}{2}$ weeks)	838
2	June 18th (Interval	July 9th 2nd-3rd round	3,038 10 $\frac{1}{2}$ weeks)	873
3	August 20th (Interval	September 10th 3rd round to December 31st, 18 weeks)	3,014	833

NB—Average amount of insecticide used per room 34.6 fluid ounces

1945 and indicates that an average of 34.6 fluid ounces of insecticide was used per room. The cubic capacity of the average room was 1,289 cu ft giving a total sprayed area of 500 sq ft per room allowing for indirect spraying of one-third of the ceiling area in addition to direct spraying of all four walls. On this basis the insecticide was used at the rate of 6.92 fluid ounces per 100 sq ft. of surface area giving a calculated deposit of 19.3 mg DDT per sq ft.

(iv) *Resulting Effects on Mosquito Infestation* (a) *All species* Table VIII and Fig 7 show both rainfall and the average numbers of mosquitoes collected

per 100 rooms in the main Takoradi township over a continuous 21 month period commencing in April 1944. Throughout this period the same general methods of mosquito control were employed so that the only important variant, apart from measured fluctuations in rainfall was the substitution of DDT residual spraying for twice weekly pyrethrum-kerosene treatment as from April, 1945 and its extension to certain outlying villages forming possible sources of mosquito infiltration. For the purpose of ascertaining the mosquito population under DDT conditions weekly check sprays with pyrethrum-kerosene were undertaken in approximately 200 rooms each of the five constituent

TABLE VIII.

MOSQUITO COLLECTIONS AND RAINFALL IN AKORADI TOWNSHIPS, 1944-45.

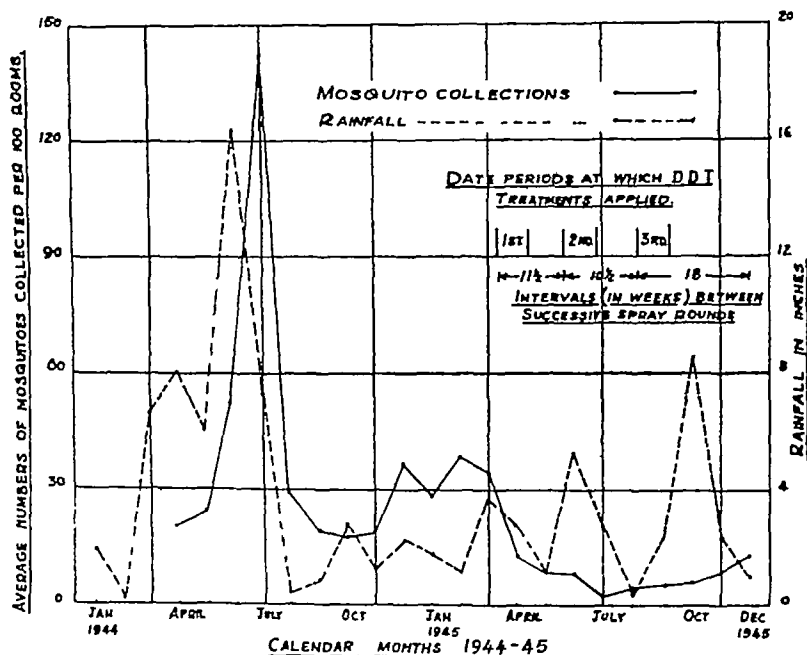
Calendar month.	Average number of mosquitoes collected per 100 rooms.		Rainfall in inches.		Number of wet days.	
	1944	1945	1944	1945	1944	1945
January	—	22	1.60	1.70	3	4
February	—	22.2	0.21	1.15	3	4
March	—	34.2	6.64	3.67	9	9
April	20.4	13.1	8.07	2.78	9	7
May	22.2	8.8	6.04	1.20	18	8
June	22.7	8.3	16.34	5.29	21	15
July	142.7	2.3	7.41	2.09	13	13
August	29.0	4.4	0.40	.41	9	8
September	19.8	8.2	0.79	2.30	12	17
October	17.6	6.1	2.69	8.49	10	16
November	18.9	8.5	1.24	.31	4	6
December	36.6	12.9	2.20	.94	8	4
Totals			63.78	32.00	113	113

zones of the township. It will be noted from Fig. 7 that not only did a considerable and immediate reduction in mosquito numbers follow the initial DDT spraying but this reduction was well sustained for 10- and 11 week intervals between successive spray rounds as also over an 18-week period subsequent to the third DDT application. Further it can be seen that under DDT conditions the numbers of all species of mosquitoes collected ceased to bear the normal direct relationship to the incidence of rainfall month by month as exemplified by results earlier in 1945 and in 1944.

(b) *Anopheles* species. Table IX and Fig. 8 demonstrate that, under DDT conditions the direct relationship obtaining under non-DDT spray killing conditions between the incidence of anopheline mosquitoes and of

FIG 7 TAKORADI AREA—DDT TRIAL V

Graph of mosquito collections and rainfall in Takoradi Township, 1944-45



rainfall was not maintained. Comparison between Figs 7 and 8 seems to indicate rather that the anopheline percentage of the mosquitoes collected increased as the total mosquito population diminished and decreased again as total collections increased thus suggesting the attainment, under DDT conditions in the present instance, of a low and relatively stable anopheline population with culicines almost entirely responsible for the fluctuations in total mosquito prevalence.

(v) *Entomology* As for Trial IV in Apowa, Staff Sergt F R P LEMMON, and subsequently his African trainees, recorded the anophelines collected as almost exclusively of *Anopheles gambiae* and *A. funestus* species, the former predominating. Among the culicines, *Culex duttoni* and *C. nebulosus* abounded but members of a variety of other species, including *Taeniorrhynchus uniformis*, *Aedes luteocephalus*, *Aed. aegypti* and *C. annulicornis*, were observed occasionally.

(vi) *Labour Organization* Regarding labour requirements, it was found that the most suitable composition for a spraying team was one recorder, one headman and eight labourers, with six of the latter handling a knapsack sprayer each and the two remaining labourers employed as carriers of reserve insecticide for refilling the sprayers. When thoroughly experienced such a team was able to spray an average of 206 township rooms per normal working day.

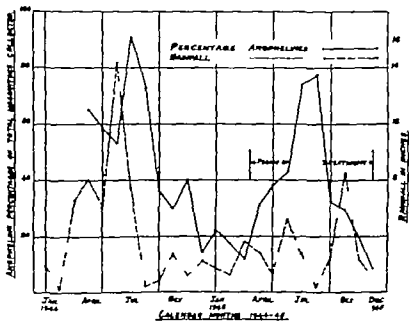
TABLE IX.

ANOPHELINE MOSQUITO PERCENTAGES AND RAINFALL IN TAKORADI TOWNSHIP 1944-45.

Calendar month.	Anopheline percentages of total mosquitoes collected.		Rainfall in inches.	
	1944.	1945.	1944.	1945.
January	—	22.7	1.86	1.70
February	—	17.4	0.21	1.15
March	—	11.8	6.66	3.67
April	65.0	30.8	8.07	3.78
May	55.6	35.4	6.00	1.30
June	63.4	43.3	16.30	5.20
July	90.9	74.5	7.41	3.69
August	73.1	77.4	0.40	0.41
September	36.4	32.8	6.79	3.30
October	29.3	29.4	2.66	8.49
November	40.0	19.7	1.24	3.31
December	14.2	8.3	2.30	0.66
Totals			53.79	22.86

FIG. 8. TAKORADI AREA—DDT TRIAL V.

Graph of anopheline mosquito percentages and rainfall in Takoradi Township, 1944-45.



(vii) *Spraying Costs* (a) *Per room* Since a team's daily wage bill amounted to 21s 6d, the labour cost per average room-spraying was 1 25d under township conditions. As regards insecticide costs, with kerosene priced at 2s 4d per gallon and DDT pulv at 4s 11d per lb, these items per township room cost 6 05d and 2 13d respectively, making a grand total cost of 9 43d per room sprayed.

(b) *Per head of population* With a population taken for comparative purposes at the figure of 10,505 persons housed at an average of 2 77 persons per room the cost of a single spraying amounts to 3 41d per head. On the basis of a single residual DDT spraying sufficing for an average of  $2\frac{1}{2}$  months, the monthly communal cost amounts therefore to 3 77d per room or 1 36d per head of population. Despite the use of expensive kerosene as the DDT vehicle these figures compare very favourably with those obtained by EDDEY (1944) using alternative spraying methods as recorded in Table X. Given availability in due course of DDT preparations capable of aqueous dilution, DDT spraying costs should be still further reduced.

TABLE X.  
MONTHLY COMMUNAL COSTS OF INSECTICIDE METHODS USED IN  
TAKORADI TOWNSHIP

Spraying agent.	Per room	Per head of population
Residual DDT	3 77d	1 36d
Pyrethrum-kerosene	11 75d	4 25d
Cresol-kerosene	12 50d	4 51d
Pyrethrum aerosol	12 10d	4 70d

(viii) *Miscellaneous Data* *Central Township of Village Sections* In the village sections of the Takoradi township area a team was able to spray an average of only 178 rooms in a normal working day of 206 rooms in the central township area. This reduction in numbers is accounted for mainly by the fact that portions of the village areas were somewhat scattered as compared with the township and a greater proportion of time was utilised in moving from one house group to another. At the same time it was found that, with an average room capacity of 1 096 cu ft and an average sprayable surface area of 456 sq ft, the village sections required 8 16 fluid ounces of 1 per cent DDT per 100 sq feet giving a calculated deposit of 22 89 mg DDT per sq ft of surface area. Spraying costs per village room under these conditions amounted to 1 45d, 2 29d and 6 51d for labour, DDT and kerosene respectively, giving a grand total cost of 10 25d. With a village section population taken at the 1943 figure of 7,162 housed on an average 2 2 persons per room

the foregoing cost amounts to 4.66d. per head of population. Monthly costs assuming a 2½ month spraying cycle, amount to 4 1d. per room or 1.86d. per head of population. Actually the dosage quoted in the present instance would seem to justify a full 3-month cycle having regard to the results detailed for the main township area using the substantially smaller DDT deposit of 19.3 mg per sq ft.

#### IV—SUMMARY

In a series of preliminary trials conducted in large numbers of native dwelling houses at Takoradi, Gold Coast Colony it was found that a 1 per cent. solution of DDT in kerosene, used to give a calculated deposit of 33.5 mg. DDT per sq. ft. of room surface, produced a marked relative absence of mosquitoes in the rooms treated. At the end of a 9-week check period following a single spraying the proportion between mosquitoes infesting the DDT'd and control rooms had risen gradually to only 4/10. When the dosage was reduced to 11.9 mg DDT per sq ft. the proportion 4/10 was reached in 13 days and complete parity with the control result ensued in a total of 28 days.

No substantial lethal advantage was obtained by substituting the "Maney" type sprayer for the "knapsack" variety and the latter was found to be the more efficient spraying apparatus for reasons given.

In a major trial conducted under semi rural conditions in a non-malaria controlled area and using a dosage of 19.75 mg DDT per sq ft., it was found that, despite its gradual increase, the DDT index factor had still to attain to its initial level at the end of 9 months from original date of spraying. Numbers of dead mosquitoes were collected in the treated rooms in the intervals between check sprayings throughout the first 10-week period.

Following adoption of the residual DDT method as a routine in Takoradi township and using a dosage of 19.3 mg per sq ft., sustained reductions in mosquito numbers were obtained over intervals of up to 18 weeks from dates of re-spraying. Further the normal direct relationship ceased between rainfall and all species of mosquitoes collected, as also between rainfall and collected anopheline mosquitoes.

Particulars of spraying costs, of the labour organization found to be most effective and of the main entomological findings are given as well as a statistical comparison of the differing results obtained in Takoradi village sections as distinct from the central township areas.

#### REFERENCE.

- ENDER L. G. (1944). Spray killing of mosquitoes in houses. A contribution to malaria control on the Gold Coast. *Trans. R. Soc. trop. Med. Hyg.* 38, 167-183.

## THE RESIDUAL ACTION OF DDT AGAINST *ANOPHELES* *GAMBIAE* AND *FUNESTUS* \*

BY

K S HOCKING, A R C S , B.S.C

### INTRODUCTION

The experiments recorded in this paper were originally undertaken to provide guidance in the practical employment of DDT impregnation as an anti-malarial measure in the East Africa Command. At the time these field experiments were begun, the information available amounted to little more than that DDT sprayed on to wall surfaces would kill mosquitoes that rested upon them, in a remarkable manner. There was an immediate need to ascertain, therefore, the optimum dosage, the effect of different wall surfaces on impregnation, and to explore, so far as the limited resources available would permit, the mode in which residual action became effective against East African vectors.

Since that time much more information has become available, much but not all of it confirming or anticipating our results. As, however, information from any angle on the use of this valuable method appears to be useful at the present stage, and as few observations on its use against *A. gambiae* and *A. funestus* have so far been published, we have thought justifiable to publish our complete results.

KNIPLING and SENIOR WHITE have published information on the residual action of DDT against other anophelids. KNIPLING (1945) has found that, used against *A. quadrimaculatus*, residual action results in a reduction of numbers, due to the restlessness of mosquitoes, which is greater than that to be expected

\* This paper is published with the permission of the D D M S, East Africa Command. Many of the observations recorded were carried out by Major D G MACINNIS, and others by various members of 92 E A Malaria Field Laboratory.



from the apparent kill that the preferred dosage is of the order of 200 mg. per sq. ft., and that a large proportion of killed anopheles have already fed.

SINIGON WHITE (1945) has pointed out the relative cheapness of this method as compared with pyrethrum spray killing.

### METHODS.

The experiments were carried out near Taveta in Kenya. Tents, huts and wooden traps were used at various stages in these trials. The tents were 180 lb. tents, 7 ft. high and with a floor area of about 14 ft. by 14 ft. Only the inner tent was impregnated. The huts were of mud and pole construction with a thatched roof, 7 ft. high and 9 ft. square inside, they were lined with four different materials—mud plaster bitumenized bessian which had been white washed, a soft compressed fibre board, and a matting made of long grass stems sewn together. The traps were of wooden frame construction 6 ft. high, and 8 ft. by 4 ft. 6 in. inside around them were panels of gauze 1 ft. deep above and below which were vertical slits 1 in. wide protected by an overhanging flap of gauze.

Impregnations of 50 100 and 200 mg. per sq. ft. in kerosene, as shown below were carried out with a power sprayer.

Morning catches were usually effected by spray killing on to white floor sheets. These sheets remained in position at night so that, before spraying all mosquitoes killed by impregnation could be separately picked up. When determining delayed mortality from residual action, as many mosquitoes as possible were hand-caught, before spray killing was carried out, and preserved in 1 ft. cube mosquito net cages. The rare survivors observed after spraying were added to the total of those caught by spraying.

Night catches were carried out (at 22.00 01.00 and 04.00 hours) by Africans and Europeans together using hand torches, after a cloth had been dropped over the end of the tent, to be raised again when catching had been completed. When mosquitoes were numerous and restless (as was observed to occur in impregnated tents) it was sometimes found impossible to catch all that were present. In such circumstances, catching was limited to 1 hour in order not to interfere too greatly with the normal nocturnal mosquito traffic.

The attachment of a second tent on to the end of the first was made in an endeavour to throw some light on the extent to which mosquitoes left a treated tent. These attached tents are referred to as annexes.

All tents and huts were "baired" by the presence of two Africans sleeping without nets—traps with one African. When annexes were attached, the bait slept in the first tent, not in the annex.

In all cases control huts and tents similarly baited, were maintained.

Some estimations of the DDT content of treated surfaces were made (by Captain R. L. HARTLEY) by estimating the labile chloride equivalent by the method of Volhard, after extraction with petrol ether.

## RESULTS

*Species composition.*—The proportion to which the observations refer were predominantly 1 *gambusia* and 1 *fundulus* in various proportions, the former being present in far larger numbers except for brief periods. The proportion of males to females of the *gambusia* was about 4 per cent, except in annexes (see below). The proportion of culicines was no more than 6 to 8 per cent.

*Rainfall.*—An analysis of the records for the distribution in mosquito population is given by the rainfall figures, and the condition of the late-year rice cultivation given in Table I.

TABLE I  
RAINFALL AND RICE

Month	Days of rain	Total inches	Month	Days of rain	Total inches
January	7	1.1	August	4	0.25
February	2	2.75	September	1	0.15
March	4	1.5	October	2	0.3
April	4	1.15	November	14	2.7
May	1	0.2	December	7	1.5
June	4	0.42			
July	2	0.10	Year		15.75

## MORNING MORTALITY AND DELAYED DEATH

The morning mortality, that is the number of mosquitoes found dead in the mosquito boxes in Tables II, III (a and b) and IV, which refer to tents, huts and trap respectively. It will be observed that the percentage of mosquitoes killed follows reasonably closely the degree of impregnation in all cases. Thus after impregnation the initial mortality given by 200 mg. per sq. ft. ranged from 72 to 93 per cent, of 100 mg. from 50 to 82 per cent, and of 50 mg. from 44 to 57 per cent.

With regard to the further mortality which might be expected to occur in those found alive in the morning, some information is given in Table V (first four columns). Of the survivors from a 200 mg. impregnated tent 44 per cent were dead within 4 hours (or 42 per cent if the mortality in the control be deducted) and 52 per cent up to 8 hours. Proportionately smaller delayed mortalities occurred in mosquitoes exposed to the tent impregnation. Even among the mosquitoes which were found in the mosquito boxes in annexes there was still a considerable delayed mortality, although it must be assumed that many of the survivors had not been exposed to a comparatively high

TABLE II

VERAGE MORNING MOSQUITO MORTALITY IN IMPREGNATED TENTS.

Ten-day period ending	A. Control total per day	B. 50 mg on 7.8.44.		C. 100 mg. on 25.11.44		D. 200 mg on 17.11.44	
		Total per day	Dead per cent.	Total per day	Dead per cent.	Total per day	Dead per cent.
1944.							
6 Dec.	60			5	29.6	13	87.0
18	50			10	31.5	24	43.4
						Reimpregnated to 200 mg	
31	233	20	34.6	22	83.7	122	83.4
1945.							
10 Jan	197	—	—	19	41.6	76	76.6
30	237	—	—	13	39.2	29	73.3
9 Feb.	244	18	31.7	18	56.2	34	77.6
19	48	18	8.2	—	—	18	67.6
1 Mar	19	5	24.0	3	—	3	—
11	8	1	—	1	—	1	—
21	3	1	—	0.5	—	3	—
31	1	1	—	0	—	—	—
10 Apr		3	—				
20		4	18.6				
30		20	26.1				
10 May		41	38.3				
20		36	41.9				
30	31	12	37.0	21	60.6	12	63.6
19 June	66	—	—	22	35.7	68	81.8
9 July	74	23	14.6	9	49.4	26	73.7
29	81	—	—	3	—	8	—
				25.6		83.2	
18 Aug	2	3	3.0	3	—	3	—
1946.							
19 Jan.	28	—	—	—	—	8	83

The persistence of this lethal effect varied much more widely and this variation seems to be connected most closely with the nature of the impregnated surface. It is in tents that the lethal effect is most persistent, little drop in effectiveness with dosages of 200 mg. and 100 mg. is apparent after 6 months, 200 mg. remaining effective for 12 months, and even from a 50 mg. impregnation some effect still remained nearly 1 year after treatment.

On mud plaster (Hut F Table IIIa) the duration is less for after 4 months there was a considerable drop in morning mortality given by a dosage of 100 mg.

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TABLE IIIA  
AVERAGE MORNING MOSQUITO MORTALITY IN IMPREGNATED HUTS

Ten-day period ending	E Control Total per day	Fibre board F 50 mg 25 11 44		Fibre board D 100 mg 20 1 45		Fibre board C 200 mg 16 12 44	
		Total per day	Dead per cent	Total per day	Dead per cent	Total per day	Dead per cent
1944	292	42	16.7			22	93.2
31 Dec						6	63.3
1945						9	68.8
10 Jan	131	27	22.0	7	81.5	4	90.9
20 "	144	47	43.8	4	55.0	3	65.4
30 "	52	21	31.5	5	26.1	4	60.5
9 Feb	109	14	24.3	2	—	1	—
19 "	65	25	8.0	4	20.0	3	55.2
1 Mar	61	9	5.5	20	5.0	9	11.1
11 "	50	12	5.8				
21 "	100	35	0				

E, F, etc. = Hut E, Hut F, etc.

N.B.—Proportion of fed in alive and dead substantially same except in Hut C and Hut D

TABLE IIIB  
AVERAGE MORNING MOSQUITO MORTALITY IN IMPREGNATED HUTS

Ten-day period ending	E Control Total per day	F 100 mg 20 5 45 Mud plaster		D Control Total per day	C 150 mg 20 5 45 On fibre board		H2 Control Total per day	H 100 mg 19 0 45 Bitumenized hessian	
		Total per day	Dead per cent		Total per day	Dead per cent		Total per day	Dead per cent
1945									
9 June	65	7	81.9	9	13	97.7	54	—	—
29 "	148	96	92.7	41	22	96.7	17	23	90.6
10 July	105	95	96.0	14	14	94.4	7	9	68.8
8 Aug	29	44	68.0	0	2	—		5	47.0
12 Sept	12	8	76.6	2	1	—		0.2	—
11 Oct	25	0	33.7					0.2	—
15 Nov	507	46	23.5					31	8.3
13 Dec	200	21	55.2					43	9.0
1946									
18 Jan	224	20	48.0				6	5	13

F, G, etc. Hut E Hut F, etc.

TABLE IV

AVERAGE MORNING MOSQUITO MORTALITY IN IMPREGNATED TRAPS.

Ten-day period ending	Control.		Wood. 1 (100 mg. on 19.9.45).		Wood. 3 (200 mg. on 9.7.45).		Whitewashed Hessian. 5 (100 mg. on 19.9.45).	
			Mosquitoes.		Mosquitoes.		Mosquitoes.	
	1	1 then 4.	Average per day	Dead per cent.	Average per day	Dead per cent.	Average per day	Dead per cent.
19 July	2	9			4	100	(Lime wash)	
29	2	5			0.3			
8 Aug	1	1			0.4			
18	0.4	0.7			0.4			
29	0.4	1			0.3			
8 Sept.	0	0.5			0.1			
18	1	0.5			0.1	72		
			<i>Impregnation here</i>				<i>Impregnation here</i>	
30	0	0.2	0	74	0.1		0.3	18
10 Oct.	0	0.1	0		0		0.1	
20	0.2	0.4	0.5		0.2		0.2	
30	5	1	1		0.4		3	
9 Nov	18	22	18	68	13	45	7	24
19	21	16	29	60	23	47	45	8
29	60	54	48	62	60	36	44	3
							<i>DDT re-moved.</i>	
9 Dec.	113	109	63	36	100	23	69	—
			49.4	67	83	33	34	—
19	103	104	48		17	30	220	—
29	44	—	22					
			48.6					
9 Jan	5	5	9	89	5	29		
19	0.4	7	11	38	6	29		

In the case of very absorbent surfaces such as compressed fibre board, the duration was still shorter—presumably due to the loss of available DDT in the thickness of the material. In Hut C a 200 mg dosage had lost much of its effectiveness after 3 months, while in the same Hut C the lethal effect of 150 mg persisted until this hut was seriously damaged by inundation. In Huts D and F the effect of 100 mg was notably less after 2 months. In Hut F 50 mg had a moderate effect lasting 2½ months.

The results from the wooden walls of the traps gave further evidence of the shorter duration of residual action on their rough and absorbent surfaces.



In them the morning mortality had dropped considerably after 4 months in the case of 200 mg., in 3 months with 100 mg.

The most notable condition, apart from the roughness or absorbency of the surface and dosage, which modified the effectiveness of residual action, was *limewashing*. The *heaman* walls of Hut H1 and Trap 5 had been limewashed, the first 3 months and the second 1 month before impregnation. It is clear that this almost completely destroyed the effect of the impregnations. The effect of 100 mg DDT applied even 3 months after limewashing lasted only 1 month, and applied 1 month after was negligible.

In Hut B, lined with *grass* matting, the results, which are not here recorded in detail, showed a morning mortality of from 20 to 25 per cent. over a period of a year. We attribute this result to the *solution* running off the smooth *grass* stems at the time of impregnation. This opinion is confirmed by a chemical estimation 3 months later which gave 5.7 mg. per sq. ft. It is, however, also to be noted that what effect existed was very persistent.

One other modifying factor is to be noted, namely the effect of rain or humidity. During May 1945 the lethal effect in Tent 6 which had previously dropped to a low level rapidly increased and then died away again. This period of heightened activity corresponded to a period of rain. We offer no explanation of this occurrence but it may be that a similar circumstance provides an explanation for the apparently rather longer duration of maximum effectiveness of the 150 mg impregnation of Hut C, during the time the surrounding fields were flooded and the immediate surroundings of the hut kept damp.

#### EFFECT ON TOTAL NUMBERS OF MOSQUITOES CAUGHT

Further reference to Tables II, III, IV shows that, accompanying the mosquito mortality observed, there was a general decrease in the numbers of mosquitoes caught as compared with the controls. It might at first sight be thought that this reduction was due to some inhibitory effect, but the results from traps (Table IV) seem clearly to contradict this explanation. When, as in these traps, the exit of mosquitoes was very difficult, the numbers caught in the treated and the untreated buildings are not significantly different. It may be noted here that the reduction in numbers caught is greatest in the case of the lighter impregnations.

This may perhaps be explained by the fact that with the lower impregnations a longer period of contact is required for the mosquito to acquire a lethal dose. Thus the restlessness induced by the DDT causes a larger proportion of exits than occurs in the higher impregnations, where the lethal dose is acquired more rapidly.

Some elucidation of the actual total entry was given by catching through the night in tents and huts. The results are shown in Table VI. It was found that the variations in such catches were surprisingly wide (in the control Hut E,

TABLE VI  
ALL NIGHT CATCHES COMPARED WITH AVERAGE MORNING CATCHES

	Treatment	Average morning catch for the period	All night catch	Morning catch after all night	Total
Tents	Untreated	13 177 8	142	47	189
	50 mg	4 40 0	108	21	129
	100 "	6 10 9	109	17	126
	200 "	3 90 8	72	19	91
Huts	Untreated	7 96 6	110	87	197
	50 mg	4 21 0	77	43	120
	100 "	2 5 4	18	12	30
	200 "	5 9 0	17	19	36

for example, between 40 and 598), and it is accordingly impossible to draw any very firm conclusions from the few series of catches made. All that can be concluded with certainty is that the increase in numbers caught by searching during the night, as compared with the morning catches for the period, is of a much higher order in treated than in untreated tents or huts. The exception is in the one case of a 200 mg treated hut, in which at this time a very high mortality was occurring. Although there was an increase in the comparative catches in the control huts, namely, 100 per cent., the increase in the corresponding treated huts was from 400 to 600 per cent. On the other hand, the results from tents, which comprise a longer series, give much stronger additional support to the suggestion that, in spite of the reduction in morning catches in treated tents, entry is in fact little reduced.

#### THE EXPLANATION OF REDUCED NUMBERS IN TREATED TENTS

The alternative to decreased entry, as an explanation of the reduced numbers of mosquitoes found in treated tents and huts, is increased exit by affected mosquitoes. That this occurs has already been suggested by the negative evidence of the numbers remaining in traps, and further positive evidence was given by the mosquito catches in annexes to treated and untreated tents, shown in Table VII, which represents the results of some seventy concurrent daily observations. Although, of course, there were much smaller numbers in the unbaited annexe than in the baited tent, the proportion in the annexe was much greater in treated than in the untreated tent.

Males, which came in near dawn for shelter, were apparently not deterred from passing through the treated tents. Similarly, unfed females were not deterred from entering, but apparently tended to pass on to the annexe in increased numbers in spite of the attraction of the bait. However, it is in the case of the fed female *Anopheles* that the most striking differences were observed.



TABLE VII  
DISTRIBUTION OF MOSQUITOES CAUGHT ALIVE IN TENTS AND ANOPHELES

Treatment	By state of feeding.				Morning catches.				Per cent. in mosquito.					
	Total.				Anopheles.									
	Anopheles.				Anopheles.									
	Culicis	Male.	Fed female.	Unfed female.	Total.	Culicis	Male.	Fed female.	Unfed female.	Total.				
Untreated	89	77	1 844	80	2,134	9	113	70	37	223	14	89.5	3.4	25.2
50 mg.	29	23	764	83	959	123	339	306	84	656	80	83.7	27.9	50.6
100	14	16	222	18	275	10	48	43	23	137	50	75.0	16.8	85.0
200	24	26	134	13	186	17	40	36	17	116	89	67.5	49.3	86.7

The very small proportion found in the annexe to the untreated tent suggests that the females normally rest in the immediate vicinity of their feeding place. Yet in spite of this tendency to remain near to where they have fed, the percentage passing into the annexe is increased in proportion to dosage up to thirteen times the percentage in the control. This occurred in spite of the fact that a large number had already been killed in the tent. It must be assumed that these females had fed in the tent, whether untreated or treated, and that in the latter case they had, for some reason, been driven out.

#### EFFECT ON BITING

Experience obtained during the course of all night mosquito catching left no doubt as to the readiness of mosquitoes to feed in impregnated quarters. When the human bait in treated tents slept under mosquito nets the proportion of fed mosquitoes fell considerably, as well as the total catch, this provides evidence not only of the extent to which *Anopheles* feed in the tent in which they are caught, but also that under these circumstances they may leave before receiving a lethal dose. The proportions of fed and unfed mosquitoes in the morning catches are shown in Table VIII. It will be noted that the proportion of fed mosquitoes caught in the various impregnations decreases but little with increasing impregnation.

TABLE VIII

PROPORTIONS OF RECENTLY FED AND UNFED MOSQUITOES IN TREATED AND UNTREATED HUTS AND TENTS

Nature of lining	Untreated		Treated 50 mg		100 mg		200 mg	
	Total females	Fed per cent	Total females	Fed per cent.	Total females	Fed per cent	Total females	Fed per cent
Tents	14,307	81.6	3,262	72.5	1,528	69.7	5,440	64.2
Wattle or mud	10,145	68.5	1,291	55.6	2,880	59.1		
Hessian	6,066	89.0			967	83.9		

#### ESTIMATION OF DDT ON TREATED SURFACES

It had been intended to make regular estimations of the DDT content of the treated surfaces, but this object was not achieved. Moreover, estimations of the results of routine impregnations in military camps showed such wide variations, in the size of sample that it was possible to examine, that such estimations give no more than an indication of the probable actual quantity present.

It was, moreover, found in general that the actual dosage as estimated was much greater for a given mosquito mortality in the case of the absorbent

surfaces of fibre board than in that of canvas. It is concluded that much of the impregnation is "buried" in the deeper layers of the fibre board.

A justifiable opinion, based on the estimations made, would be that it is necessary for at least 50 mg. per sq. ft. to be present within the superficial layers of impregnated material in order to achieve an effective residual action.

### CONCLUSIONS

It appears to us that, from the foregoing observations, certain conclusions can be drawn as to the effect of the residual action of DDT on the house populations of *A. gambiae* and *funestus*. There has been no indication of any difference in the effect on these two species.

We conclude in the first place that there is no interference with the normal behaviour of these species so far as their entry into the huts and tents and their biting activity is concerned. When, however, they have been affected by contact with DDT they tend to leave the building concerned, and as a result a reduction in numbers of mosquitoes, greater than that attributable to apparent mortality, is found in it.

The mortality from adequately impregnated surfaces, either during the night or later, is certainly very high, and probably closely approaches 100 per cent. provided the mosquitoes are able to feed. If however they cannot feed, or if there is an unimpregnated surface in the immediate vicinity on which they can rest after feeding, it is likely that a considerable number will escape before coming into contact with the impregnated surface.

Reduction in malaria transmission will not be achieved by mosquito mortality, however great, in a single impregnated building: to ensure this reduction similar treatment must be given to all human habitations in the neighbourhood, probably up to a radius at least as great as that found necessary in larval control of the anopheline species concerned. It cannot be expected that this method will be more than very partially effective as an anti-malarial measure against species which do not habitually rest in houses. Nor in a single house or small group of houses, can this method be expected to be more effective than spray killing, although it is likely to be much cheaper.

The dosage which may be regarded as most effective is open to discussion. The observations which are particularly relevant are those concerning morning mortality with varying dosage, together with the delayed mortality in annexes to treated tents. In the light of these findings it seems to us that the higher range of dosages used, namely 150 to 200 mg. per sq. ft. is necessary in order to achieve an adequate kill, that is one in the region of totality. A necessary conclusion from the results given seems to be that, unless knock down is rapid, escapes will be considerable.

Although we have no precise indications of the relationship between dosage and the nature of surfaces in general, it is at least clear that a thick, rough absorbent material requires a much higher dosage in practice than a

than one of finer grain. As we found, the same dosage actually remaining on the material is initially less effective, and its duration less, on the former type of material than on the latter.

As the fibre board we used is probably as absorbent as any material likely to be encountered in buildings, it may confidently be expected that, on any surface, an effective duration of at least 4 to 6 months will be ensured by an impregnation of 150 to 200 mg per sq ft, 100 mg per sq ft dosage cannot be expected to retain its effectiveness for more than half that length of time.

Lime wash, particularly if recently applied, effectively neutralizes the residual action of DDT on walls.

#### SUMMARY

The methods used to investigate the residual action of DDT are described.

The original mosquito mortality caused, and the duration of the effect, with various dosages of DDT on various surfaces are recorded.

The reduction in the numbers of mosquitoes found in treated tents and huts is discussed.

The large proportion of the mosquitoes killed that have already fed is recorded.

It is concluded that *A. gambiae* and *A. funestus* tend to leave a lightly treated building, but that with an impregnation of about 200 mg per sq ft final mosquito mortality approaching 100 per cent will be achieved, and that on most surfaces the effect will last for at least 4 to 6 months unless lime is present.

Impregnation at this dosage on a sufficiently wide scale should greatly reduce malaria transmission by *A. gambiae* or *A. funestus*.

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## EXPERIMENTS WITH DDT ON VARIOUS SPECIES OF TSETSE FLIES IN THE FIELD AND LABORATORY

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### INTRODUCTION

SWYNNERTON and many other tsetse workers had considered the possibilities of an insecticide which fitted with the following requirements for controlling and exterminating tsetse flies —

- (a) Must have lasting effects
- (b) Able to kill tsetses after a short contact
- (c) Must not be readily destroyed by sunlight or heat.

No insecticide fitted these requirements before the properties of DDT were generally made known. The only insecticides that gave any promise at all were solutions of pyrethrins, these were lethal to tsetses which came in contact with a sprayed area, but only while the solution remained, and the pyrethrins were quickly destroyed by sunlight. HORNBY (1943) successfully used solutions of pyrethrins as a tsetse repellent the solution did not repel tsetses in the accepted use of the word, since the same numbers of flies were attracted to both treated and untreated baits, but the treatment deterred the tsetses from biting and so infecting (with trypanosomiasis) the treated baits.

\* I wish to thank Mr S NAPIER BAX, Acting Director, Mr W H POTTS, Dr C H N JACKSON, Senior Research Officers (Entomologists), for their help, suggestions and co-operation in this work, also Professor J E HARRIS and Dr E T BURTT for their help in the preparation of this paper. The careful work of many African assistants is also gratefully acknowledged.

In fact actual contact was needed with the treated surfaces before the flies were repelled, and I suggest that substances which repel after contact should be called contact repellents to avoid confusion.

We received a small supply of DDT in November 1944 and during early 1945 ample supplies through the kindness of Professor P. A. Buxton, and the London Tsetse and Trypanosomiasis Committee. With these supplies I was able to carry out a series of preliminary experiments on various species of tsetse both in the laboratory and field. It was quickly demonstrated that contact through the pulvilli of the feet for a few seconds with a sprayed surface containing DDT either fresh with solvent or as a residual deposit, was extremely lethal to all species of tsetse tried. In fact DDT had all the desired qualities as listed above. For details of the properties of DDT see Buxton (1945).

The next problem was how this insecticide could be used most economically bearing in mind that our object is not merely the control of the fly but its extermination from any given area. There are already several methods for controlling and exterminating tsetse in given areas and since vast areas of Africa are dominated by the pest, the cost of ridding these areas is the main consideration when deciding which method should be used. The tsetse lives singly in forested areas, feeding solely by sucking blood of animals (including man) and in so doing may transmit the trypanosomes of human sleeping sickness (*Trypanosoma rhodesense* and *T. gambiense*) or animal trypanosomiasis (*T. congolense*, *T. brucei* and *T. evansi*). Human sleeping sickness can be dealt with fairly easily in East Africa by removing the people from infected areas (see FAIRBAIRN 1944) but the presence of tsetse prevents the development of the land and expansion of the already overcrowded population. The situation has been described admirably by Bax (1944).

During the war the military had been able to dust large areas of bush, forest and swamps from the air controlling, and in some cases exterminating, mosquito species from certain areas in Burma. Such a measure against the tsetse was considered however the cost of the DDT is at present about 4s. 6d. per lb., and even if it could be produced at 1s. per lb. the cost of spraying from the air would be prohibitive even if successful. It would require about  $\frac{1}{2}$  lb. per acre to be at all effective, or 320 lb. per square mile which, at 1s. a pound, would cost £16 per square mile for DDT alone. Experiments with DDT in the bush tend to show that it is only effective for 4 to 5 days on vegetation since it appears to be absorbed by certain plants. Assuming that one aerial dusting destroyed all the adult tsetse, the pupae still remain in the ground and these will continue to emerge for the next 30 to 35 days at mean temperatures of 24° C. and 22° C. If a second dusting was given 35 days later some flies that emerged soon after the first dusting had become non-lethal would have had time to deposit their first larvae (20 to 22 days being required at mean temperatures of 24° C. to 22° C.). So at least three

aerial dustings would be required, costing approximately £48 per square mile in DDT alone (at the present price this would be £216). This method, if successful, would not be economical since there are several other cheaper and more certain methods for exterminating tsetse. This applies to all methods of applying dusts or sprays to the bush in general.

Another method that commanded our attention was the possibility of making cheap tsetse traps sprayed with DDT and placed about the bush. Many types of tsetse traps have been invented to date and although many are very efficient and large-scale attempts have been made to "catch out" tsetse in given isolated areas, none has so far succeeded nor proved economical. Details of tests carried out with DDT in this connection are given in the paper, but this line of investigation has been left in abeyance, since it would prove more expensive and did not appear so hopeful as the method described below.

Experiments have shown that cattle are a favoured host of *G. pallidipes* Austen (VANDERPLANK, 1944) and cattle are also a favoured host of *G. swynnertoni* Austen, *G. morsitans* Westwood, *G. austeni* Newstead and some members of the *fusca* group. It was thought that if cattle could be dipped in or sprayed with a solution, suspension or emulsion containing DDT, which was lethal to tsetse as a residual film, then the area in which tsetse are to be exterminated could be "flooded" with cattle, the numbers used being in excess of the game present, which seldom exceeds 100 per square mile. Assuming that the numbers of treated cattle and untreated wild game were equal, and no preferences were shown by the tsetse for the game, then the chances are that the tsetse would attempt to feed off the cattle every other meal and would be poisoned by the DDT. The presence of large numbers of cattle in an area tends to frighten the wild game away, especially the larger species which are most preferred as hosts by members of the *morsitans* group. During 1940, using only three oxen and nine African assistants, I have been able to mark up to one-third of the tsetse (*G. pallidipes*) population in a fortnight, in an area of  $2\frac{1}{2}$  square miles (6,500 were marked and at the end of the period one in every three flies caught had been marked) so it seems feasible that, using as many as seventy-five cattle to the square mile, it should be possible to account for the whole population in 8 to 10 weeks. A female emerging from pupae, to exterminate the population, requires a minimum of five meals before she produces her first larva, the time taken to produce this larva and the interval between each meal depends upon the prevailing temperature, but the number of meals required appears to be more or less constant (VANDERPLANK, unpublished). If conditions are such that she is most likely to attempt to take one or all these meals off the treated baits, she will be poisoned and killed before she is able to reproduce. Any method which controls or merely reduces the numbers of tsetse in an area is not satisfactory since one infected tsetse can do nearly as much damage as a relatively high population, hence, as already stated, our aim is the complete extermination of tsetse from any given area.



Experiments then, have developed along the lines of obtaining a suitable preparation of DDT which can be placed on cattle and then using them to attract and so destroy the tsetse flies. Incidentally this method destroys other obnoxious biting flies, also hard ticks, thus serving more than one purpose at the same time this method does not destroy any useful insects or predators of tsetses, which spraying the bush mechanically would do. The chief predators of tsetses that are susceptible to DDT are many species of robber flies (*Aniba*), wasps (*Bombes*) mantids, *Alukia* species, ants, tree-frogs, and small tree lizards. Predators that are not affected by DDT are birds, small mammals (preying on pupae), and spiders, none of the last, however plays an important part in controlling the tsetse population.

#### LABORATORY EXPERIMENTS.

The first experiments were made to determine whether DDT was lethal to tsetses and in which way the DDT must be brought into contact with the flies. In the first experiments, pieces of cardboard sprayed with 5 per cent. DDT in kerosene were introduced into Bruce boxes\* containing twenty to thirty *G. morsitans*, *G. swynnertoni* and *G. pallidipes*. All the flies made contact with the strips of paper and were dead in 24 hours. The next experiments were aimed at determining the duration of contact necessary with different strengths of residual DDT. Single tsetses in gauze-covered tubes were placed on sprayed strips of paper for periods of 1 to 5 minutes, but the gauze interfered and prevented the flies coming into contact with the treated area and the majority survived. Next experiment six *G. palpalis fuscipes* and five *morsitans* were allowed to feed through cotton gauze (flies kept singly in tubes) on an ox which had been sprayed some hours previously at the rate of 0.5 grammes of DDT (active isomer 60 per cent.) per square foot. All died within 24 hours. Controls were three *palpalis fuscipes* and two *morsitans* which were fed in a similar manner on an untreated ox—all were alive and well after 30 hours. The conclusion is, in the light of later work, that flies were willing to feed through a treated surface providing that their feet did not contact the poison, and were killed within 24 hours through DDT contacting their proboscis, 0.3 gramme active isomer of DDT per square foot being lethal. This experiment was repeated 24 hours later on the same oxen. Eight male and three female *palpalis fuscipes* and one male *austeni* were used, most were paralysed within half an hour and dead within 12 hours but two *palpalis fuscipes* took 48 hours to die. Three male *palpalis fuscipes* and two male *morsitans* were used as controls and all were alive and well after 48 hours.

Experiment 4 was a repeat of the above, but 72 hours after the spray had been applied the area treated was on the flanks of the ox, and this ox was grazed with its herd when not in use. The control ox was grazed and housed separately.

A Bruce box is a small gauze-covered box, similar to those described and used by Bruce in his work.

to avoid contact with any treated beasts. Four male and four female *palpalis fuscipes*, two male and two female *morsitans*, one male *G. fuscipleuris* Austen and one female *G. brevipalpis* Newstead (the only tsetse flies available at that time) were used. At the end of 24 hours one male and two female *palpalis fuscipes* had died and the remainder survived and were well after 48 hours. Controls were one male and two female *palpalis fuscipes*, two male and one female *morsitans*, of which one female *palpalis fuscipes* died within 24 hours, the rest were alive and well after 48 hours.

The conclusion is that as far as "proboscis contact" is concerned this method had no effect 72 hours after spraying. Later it was shown that this is due to the residual crystals of DDT becoming rubbed off the ox by the action of grass, shrubs, etc., and the cattle rubbing against one another.

**Experiment 5.** An emulsion (formula SK/4, see Appendix) containing DDT was sprayed on a piece of ox hide at the approximate rate of 1 gramme per square foot, this is a heavy dosage but was due to the thickness of the hairs on the hide absorbing the spray. If the total surface area of the hairs plus skin could be calculated the dosage per unit area would be considerably lower. The tsetse flies were placed in contact without any intervening gauze for a set time, being held by the wings with a pair of forceps. A male *pallidipes*, male *swynnertoni*, male *morsitans* and a male *palpalis fuscipes* were held so that their feet were in contact for 20 seconds, all were paralysed within 15 minutes and dead within 2 hours. The same species were used for controls, and used alternately with the experimental flies, using the same forceps, but a clean piece of ox hide and all of these were alive and well after 30 hours. In the same experiment a female *palpalis fuscipes*, a female *morsitans*, a female *swynnertoni* and a female *pallidipes* were held in contact with the treated surface for 15 seconds, all were paralysed in 20 minutes and dead within 2½ hours. Controls of the same sex and species were living and well 30 hours afterwards. Similarly a number of each species were held in contact for 10 seconds and these were paralysed in 30 minutes and dead within 4 hours, another set were held in contact for 5 seconds each and these were paralysed in 90 minutes and dead within 6 hours. Each treated fly had its equivalent control, all of which survived and were well 30 hours afterwards.

Some thirty experiments of this nature were carried out by myself, involving 531 individual flies of various species. The species used were *palpalis fuscipes* from Uganda, *palpalis martinii* (Zumpt) from the neighbourhood of Abercorn, Lake Tanganyika, *morsitans morsitans*\* from Kondoa Irangi, Central Tanganyika, *morsitans orientalis*\* from Kingolwira, Tanganyika, *swynnertoni* from Shinyanga,

\* The writer found that *morsitans* from the Central Fly Belt, Tanganyika, when crossed with *morsitans* from the Coastal Fly Belt, Tanganyika, produces sterile male hybrids, and subsequently slight morphological and physiological differences between these two races or subspecies. In view of these differences I propose to publish and use the trinomials as given here. *G. morsitans morsitans* is the typical race as described originally by Westwood.

*pallidipes* from Shinyanga, *fuscipennis* and *brevipalpis* from Kisii Kenya, *longipennis* from Mbulu, Tanganyika, and *austeni* from Kingolwira, Tanganyika.

The procedure in these experiments was as described for the experiment above, namely each fly was held with its feet in contact with the surface. Experimental and control individuals were tested alternately contact time was gauged with a stop watch by a second observer Mr W. H. POTTS. In this way several "home made" emulsions in various strengths were tested tests were also carried out with pyrethrum extracts. The detailed experiments are too lengthy to be inserted here even in table form, and it is proposed to summarize the findings and conclusions of such experiments. Copies of the original data are filed at the Tsetse Research Department's Headquarters, Tanganyika whence copies could be obtained. During 1946 at the Zoology Department, Bristol University DDT has also been found to be equally lethal to *G. p. palpalis*, *G. tachinoides* and *G. submorsitans* from Kaduna, Nigeria.

If solutions of DDT in kerosene, diesel oil, carbon tetrachloride, chloroform or other volatile solvents were used, the solvent quickly evaporated after being sprayed upon the surface leaving needle like crystals of DDT which could be readily observed under the microscope. The size of these crystals varied with the strength of the solution of DDT the solvent, and the time taken to evaporate. If the DDT dissolved in a solvent was then emulsified, the size of the residual crystals depended upon the size of the particles of the disperse phase of the emulsion and the strength of the DDT dissolved in it. When all the disperse phase particles were between 1 and 7 microns in diameter as with our emulsions, a 5 per cent. DDT solution in kerosene gave long needle-like crystals and a  $\frac{1}{2}$  per cent. DDT solution in kerosene gave short rectangular crystals. The space between these crystals depended on the amount sprayed on a given surface area and the proportions of continuous to disperse phases of the emulsion. It was found that the effectiveness of the DDT depended roughly on the amounts picked up by the pulvilli of the insect's feet, and that it was more advantageous to have numerous small residual crystals over the surface than a few long crystals although there may be many more milligrams of DDT per unit area on the long crystal surface than on the short crystal surface. A high percentage solution of DDT emulsified with several times its volume of continuous phase (water base) gives irregular results when attempting to determine the minimum lethal dose in relation to contact time presumably some flies happen to contact a large crystal and become poisoned while others are fortunate enough to avoid them. On the other hand, a low percentage solution of DDT gives a more definite end point when determining the minimum lethal dose in relation to contact time presumably because the fly has to come in contact with several of these smaller crystals before it receives a lethal dose and is not killed if it contacts only one or two crystals.

The general conclusion drawn from the experiments, which were by no

means complete, was as follows on dried ox hide, a  $\frac{1}{4}$  gramme per square metre applied as a  $\frac{1}{4}$  per cent solution of DDT in kerosene, emulsified with equal parts of gum solution or some other adhesive, was as effective as 5 grammes per square metre applied as a 5 per cent solution in kerosene, emulsified with equal parts of gum solution, and a  $\frac{1}{4}$  gramme per square metre applied as a  $\frac{1}{4}$  per cent solution was far more effective than diluting the 5 per cent DDT emulsion (or spraying less over the surface) so that a  $\frac{1}{4}$  gramme was distributed over the square metre.

In the laboratory the residual effect lasted from 6 to at least 12 weeks, the higher the percentage of DDT in solution the longer the effect lasted, that is to say that DDT is either absorbed or vapourizes slowly, and the larger residual crystals last longest, this counteracts the previous conclusion when deciding what strength should be used. A highly volatile solvent is preferable to a non-volatile DDT solvent for work with *Glossina*. A very high percentage of DDT must be dissolved in paraffin wax before the mixture becomes effective, similarly for petroleum jelly (vaseline) and heavy oils. The chief objection to heavy or non-volatile oils is that when they are emulsified and sprayed on to a surface, the water base (continuous phase) evaporates first, leaving a thin oil film, whereas in our work it was desirable that the DDT solvent (disperse phase) should evaporate first, leaving the DDT crystals to be stuck to the animal's hairs by the adhesives in the water-base (continuous phase). This was the case when the DDT was dissolved in kerosene, chloroform, carbon tetrachloride and other volatile solvents, and then emulsified.

It was also discovered that the different species varied in their degree of sensitivity to DDT. *G. pallidipes* was the most sensitive, followed by *G. morsitans*, both sub-species, *G. swynnerton*, *G. austeni*, *G. palpalis fuscipes*, *G. palpalis martini*, *G. fuscipleuris*, *G. longipennis* and *G. brevipalpis*. *G. palpalis fuscipes* required about twice the minimum lethal concentration or contact time as *G. pallidipes*, and *G. fuscipleuris*, *longipennis* and *brevipalpis* about twice that of *G. palpalis fuscipes*, or four times that of *G. pallidipes*. This sensitivity appears to have no obvious morphological basis, the pulvilli appear to be relative to the size of the species, and the species listed in order of their size, commencing with the smallest, are *austeni*, *swynnerton*, *morsitans orientalis*, *palpalis fuscipes*, *morsitans morsitans*, *palpalis martini*, *pallidipes*, *fuscipleuris*, *brevipalpis* and *longipennis*. The relative sensitivity may be due to the nature of the epidermal waxes, and awaits further investigation.

Experiments with pyrethrum extracts showed that while the solvent was still present they were extremely lethal, and had an immediate "knockdown," but no residual effect. However, kerosene alone had a lethal effect even through pulvilli contact. A solution of sodium arsenite also has a lethal effect through the pulvilli and especially if the fly probes the poisoned surface. The details of the sodium arsenite experiments will be given in a separate paper.

Although DDT has no repellent effects in the accepted use of the word,

It is definitely a contact repellent for all *Glossina* species so far tested. A few seconds after the tsetse has been in contact with DDT on the treated surface, its feet appear to become irritated in such a manner that its normal probing reflex is inhibited, and instead it rubs its legs together attempting to clean the irritating particles off, first one pair then the next, and then either moves to another spot on the host animal or flies off—however during its 1 to 5 second contact it has received sufficient poison to be killed. DDT has no lethal action on either *Trypanosoma rhodesiense brucei congolense* or *trux* whereas HORNBY has shown, and the writer confirms, that pyrethrum extracts are trypanocidal but harmless to mammals even when injected (aqueous or alcoholic extracts) in relatively large quantities. Although DDT has no immediate toxic action on mammals used in this work, prolonged application may cause chronic poisoning. It is extremely difficult to get tsetse to feed on animals that have been well coated with DDT unless the flies are separated from actual contact (except the proboscis which appears to be insensitive) by cotton gauze.

#### FIELD EXPERIMENTS.

The main object of the preliminary field experiments was to discover a suitable preparation of DDT which could be applied to the bait animals and remain lethal to tsetse as long as possible. The method was as follows: two teams of trained bait oxen were chosen at random. One team was treated with DDT preparation that was to be tested, and the other team left as a control similarly two teams of Africans with black cloth attractant screens were chosen at random, the screens of one team being treated and the others left as controls. To avoid any possible contact between either men, baits or screens, the laboratory was divided into two parts with separate entrances, the DDT treated screens and their teams and treated ox teams using one part, and the control teams used the other part, as their assembly and return base. The two groups of oxen were housed and grazed separately when not working. The experimental baits were sprayed with the preparation on the 1st day only and then the experiment continued until no further effect of the treatment was detected. All parties went out into the bush 1 mile away and wandered at random catching all tsetse *G. pallidipes* and *G. morsitans* that alighted on the baits, marking them with predetermined colours and releasing them, recording the numbers, sexes and species marked. Any marked tsetse encountered, having marks other than those being used by any of the parties on that day were killed and brought into the laboratory. Marked flies were also recaptured by parties engaged on routine fly rounds and on other experiments being carried out in the area. All these marked flies were brought into the laboratory and counted in the results. Since the numbers of parties varied daily this has exaggerated the differences in the recapture percentage from week to week. The numbers of marked tsetse recaptured depended on several factors, such as population, number marked, parties engaged in recapturing and the interval of time allowed to lapse between

marking and recapture, but in this case these factors were equal for all parties engaged, both treated and controls

Consideration was also given to the possibility that marked tsetse flies are more conspicuous to predators than unmarked, this varies with the colour used and season. To avoid any bias each fly was marked with two or three colours. Consideration had to be given also to the possibility of the African assistants making errors, although the system used was practically foolproof and so arranged that if any mistakes were made they could be detected on receiving the recaptured flies. Since the tsetse flies had to be marked with two colours or more, if any flies had been recaptured with only one mark (and even if the colour had been rubbed off, traces could always be detected under the microscope) the whole experiment would have been deemed invalid. The assistants were issued only with the two or three particular colours for that day and their own particular team of baits.

If the DDT treated baits had no effect on the tsetse flies there would be no significant differences between the numbers recaptured from them and the controls, on the other hand, if the treated baits killed all the tsetse flies alighting on them then none would be recaptured and so the differences in the percentage recaptured from treated and control baits indicated the effectiveness of the DDT preparation after due allowance is made for mathematical probabilities.

This method worked very well in practice and the experiments were continued until there were no significant differences between recaptures from the control and experimental baits. Another precaution that was taken was to issue periodically, generally every other day, clean nets to the assistants with the treated baits to prevent the nets becoming contaminated and so biasing the results.

Table I gives the results of the first experiment, which compares the recaptures from screens treated with 30 grammes of DDT (active isomers 60 per cent) which had been dissolved in carbon tetrachloride as a 5 per cent solution, and hand sprayed on the screens. The carbon tetrachloride evaporated very quickly, before the screens had left the laboratory, and left needle-like crystals of DDT intermingled in the cotton cloth. On day 0, the day the screen had been treated, some 15 per cent of the untreated (control) screen-caught tsetse flies were eventually recaptured whereas only 5 per cent of the treated screen-caught tsetse flies were recaptured. The difference by using the original data is significant, and the DDT treatment is rated as 66 per cent effective on that day. On the 2nd day, that is 48 hours after spraying, the treated screens were only 50 per cent effective, but significantly so, on the 3rd day there were more recaptures from the treated screens than from the controls, and the DDT was no longer effective. It appeared from microscopical examination of the treated screens that the crystals of DDT had come off, due probably to the friction of the material swaying as carried and possibly brushing against the bush and grass. Other pieces of the same material kept

TABLE I.

COMPARING UNTREATED SCREENS WITH SCREENS SPRAYED WITH 30 GRAMS OF DOT (50 PER CENT. ACTIVE ISOMER) AS A 5 PER CENT. SOLUTION IN CARBON TETRACHLORIDE.  
MALE AND FEMALE *G. trypanotomus* AND *G. pallidipes*.

Days after original spraying screens. (1)	Control screens.			Treated screens.				$\chi^2$ (8)
	Numbers marked. (2)	Numbers recaptured. (3)	% recaptured. (4)	Numbers marked. (5)	Numbers recaptured. (6)	% recaptured. (7)	% effective. (8)	
0	443	70	15.1	207	11	5.3	66	Sig.
2	229	80	31.8	142	15	10.6	50	
3	221	28	11.3	160	20	12.5	0	Not sig.

Column 8 gives the approximate percentage of tsetse killed by the treated screens and Column 9 the result of testing whether the differences in recaptures between the control and experimental screens have any mathematical significance.

In the laboratory still had their DDT crystals and were still lethal to tsetse. From this experiment it appeared that it was necessary to find some way of sticking the DDT crystals to the screens and other baits.

To serve this purpose an emulsion (formula SK/4) was devised. Fifty parts of a 5 per cent. commercial DDT (active isomer 77 per cent.) dissolved in kerosene was emulsified with fifty parts of fresh ox serum to which 0.5 per cent. sodium arsenite had been added as a preservative. The kerosene or disperse phase carried the DDT and was broken up into globules between 1 and 12 microns in diameter average size being 7 microns and the continuous phase acted as the adhesive. When a thin layer of this emulsion evaporated, the kerosene fraction evaporated first, leaving fine crystals of DDT which stuck to the drying serum. This emulsion was tested in the laboratory also on screens and bait oxen in the field. Tables II and IV give the field results for bait oxen and screens. On bait oxen, Table II, it was 92 per cent. effective on day 0 and 100 per cent. effective on the following, day 1 and gave significant results up to and on day 5. On day 6 however it was only 45 per cent. effective, this result not being significant, and may be due to chance as on day 9. Although the experiment was continued up to the 18th day there were no significant differences after the first 5 days between the recaptures of tsetse marked off the control and treated oxen, in fact in some cases more recaptures were obtained from those marked off the treated baits than from the controls, but not significantly more. So it can be concluded from this experiment that the emulsion was effective for about 5 or 6 days (6 days if day 0 is included) after which the effect is lost.

The laboratory treated pieces of skin remained 100 per cent. effective 3

months after the original application, so it appears that the loss of effectiveness on the living oxen is due to some action concerned with the living animal. It may be absorbed by the hairs or skin of the living animals or what is more likely is that it is rubbed off by the cattle rubbing up against one another, by friction with passing grass (grass grows up to 6 ft or more in these areas) and bushes, also when the animal lies down to rest.

TABLE II

COMPARING UNTREATED OXEN WITH OXEN SPRAYED WITH AN EMULSION 5K/1 (SEE APPENDIX) EACH OX RECEIVING APPROXIMATELY 10 GRAMMES DDT (70 PER CENT ACTIVE ISOMER)  
MALE AND FEMALE *G. styannertom* AND *G. pallidipes*

Days after spraying (1)	Control oxen			Treated oxen				$\chi^2$ (9)
	Numbers marked (2)	Numbers recaptured (3)	% recaptured (4)	Numbers marked (5)	Numbers recaptured (6)	% recaptured (7)	% effective (8)	
0	57	14	24.6	105	2	1.9	92	Sig
1	72	11	15.3	156	0	0.0	100	"
2	82	10	12.2	99	2	2.0	84	"
3-4	No catches							
5	79	7	8.5	42	1	2.4	72	Not } Sig
6	149	13	8.7	62	3	4.8	45	
7	235	21	8.9	98	4	4.1	54	
8	108	8	7.4	73	8	11.0	0	" sig
9	158	10	6.3	91	2	2.2	65	" "
10-11	No catches							
12	135	31	23.0	117	29	24.8	0	" "
13	107	19	17.8	84	12	13.2	—	" "
14	84	16	19.2	79	14	18.2	—	" "
15	135	22	16.3	63	13	20.8	—	" "
16	54	5	8.5	87	12	13.2	—	" "

Column 8 gives the approximate percentage of tsetse killed by the treated screens. Column 9 the result of testing whether the differences in recaptures between the control and experimental oxen have any mathematical significance. If days 5, 6 and 7 are combined the DDT treatment is 59 per cent effective and the differences between treated and control oxen are significant ( $\chi^2 = 5.5$ ).

Table IV shows the results of testing the emulsion on cloth attractant screens, and since the screens were less exposed to any friction they were effective for a much longer period. However, the emulsion was only 100 per cent effective on one day, the 2nd day after application. It was found that the average time a tsetse was allowed to settle on the screen before being caught was 3 to 5 seconds, this is a far shorter period of contact than would occur if the tsetse was allowed to stay and attempt to feed or merely "look round".



throughout the whole experiment. No attention was paid at the time to climatic conditions, but on looking up the meteorological records, it appears that the screens were less effective when humid conditions existed—thus, of course requires further investigation. On days 33, 34 and 35 the treated screens were losing their effectiveness as confirmed by laboratory tests. So under suitable weather conditions this emulsion is between 60 and 100 per cent. effective in killing *myrmecotus* and *pallidipes* for 30 days after application on screens. This is much longer than its duration of effectiveness on bait oxen, the reason no doubt due to differences in surface wear and tear on the two baits.

In the next two experiments, which were run concurrently an emulsion, formula GD/10 was tested. The essential difference between this emulsion and the last is in the nature of the oil used in the disperse phase—here diesel oil was used instead of kerosene and a 5 per cent. gum solution instead of serum in the continuous phase. Since the diesel oil was not so volatile as kerosene the continuous phase evaporated first and left an oily film over the gum crystals, defeating the principle of the adhesive, which was intended to stick the DDT crystals to the surface. These results are shown in Tables III and V. Considering recaptures from the oxen, if daily comparisons are taken a significant result was only obtained on the same day as the beasts were sprayed and on days 7 and 8 subsequently. This is due to the small numbers of flies that were marked on the control and treated animals. However if the results are taken at weekly intervals a significant result is obtained for the 1st and 2nd weeks, but no effect was observed for the 3rd week—in fact, slightly more flies were recaptured from the treated oxen than from the controls in this period. The emulsion was 73 per cent. effective during the 1st week and 60 per cent. during the 2nd. This was rather better than the results obtained with the previous emulsion and shown in Table II. Table V shows the results obtained from the control and treated screens. If daily comparisons are made, numbers are sufficient to give significant results on all except 3 days. From meteorological data, it appears that on the 3 days which do not give significant results it was wet or rain had fallen during the previous night. Laboratory tests with three to five flies given 5 seconds' contact with these treated screens after use, gave 100 per cent. kill throughout the whole period, providing the screens were dry. If however the screens were wet the effects of the DDT were reduced. The film of moisture appeared to provide some insulation between the fly and the poison, and may have prevented to a large extent the fine crystals of DDT breaking out of the surface and sticking to the fly's feet.

Taking these results on a weekly basis, 79 per cent. of the flies were killed during the 1st week, 57 per cent. during the 2nd, and 76 per cent. during the 3rd, after which the experiments were discontinued.

Table VI shows comparative recaptures from both types of controls used in the various experiments. The numbers recaptured from the screen and oxen seem to vary between each other on the same day and between themselves from

TABLE V

COMPARING THE MARKED RECAPTURES OF *G. steynertoni* AND *G. pallidipes* FROM UNTREATED (CONTROL) SCREENS AND SCREENS TREATED WITH DDT EMULSION (FORMULA GD/10 OF 5 3 45) APPLIED AT THE RATE OF 5 GRAMMES DDT (ACTIVE ISOMER 77 PER CENT) PER SCREEN OF APPROXIMATE SURFACE AREA OF 2 SQUARE METRES

Date of experiment from 5 3 45 to 24 3 45 inclusive

Days after spraying (1)	Control screens			Treated screens				$\chi^2$ (9)
	Numbers marked (2)	Numbers recaptured (3)	% recaptured (4)	Numbers marked (5)	Numbers recaptured (6)	% recaptured (7)	% effective (8)	
0	300	27	9.0	195	4	2.1	78	Sig
1	242	36	14.9	227	6	2.6	83	"
2	190	29	15.3	116	4	3.4	78	"
3	297	34	11.4	201	3	1.5	87	"
4	186	21	11.3	198	1	0.5	96	"
5*	487	57	11.6	151	11	7.2	38	Not sig
Week 1	1,704	204	12.7	1,088	29	2.7	79	Sig
7	170	30	17.6	168	11	6.5	65	Sig
8	142	17	11.9	186	17	9.1	24	Not sig
9	194	36	18.6	223	15	6.7	64	Sig
10	150	29	19.3	187	13	7.0	64	"
11	185	29	15.6	267	5	1.9	88	"
12	551	40	7.3	155	5	3.2	56	Not sig
Week 2	1,392	181	13.0	1,186	66	5.6	57	Sig
14	507	46	9.1	237	2	0.8	92	Sig
15	247	23	9.3	190	5	2.5	73	"
16*	403	36	8.9	138	6	4.4	50	Not sig
17	317	36	10.4	121	2	1.6	85	Sig
18	332	43	12.9	154	5	3.2	75	"
19	390	58	14.5	157	6	3.8	74	"
Week 3	2,205	242	11.0	907	26	2.6	76	Sig

\* Wet days

day to day, but testing these differences by  $\chi^2$  only on two occasions would these differences appear to be significant. Actually this is not the case since in the large number of random samples one would expect to obtain a so-called significant difference by chance. When the tables are summed up and compared, there is no significant difference between the percentage recaptured off the screen or off the oxen.

TABLE VI.

COMPARATIVE RECAPTURES FROM BOTH TYPES OF CONTROLS. RECAPTURES OF FLIES MARKED BY UNTREATED SCREENS AND UNTREATED GRASS, MARKED ON THE SAME DAY AND IN THE SAME AREA, BUT WITH DIFFERENT COLOURS. COMPARATIVE SELECTION FROM TABLES II, III, IV AND V

MALES AND FEMALES *G. sayoniensis* AND *G. pallidipes*.

Date.	Control screen.			Control grass.			Difference by $\chi^2$
	Numbers marked.	Numbers recaptured.	% recaptured.	Numbers marked.	Numbers recaptured.	% recaptured.	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
16.2.48	169	22	13.0	83	10	12.0	Not sig.
19.2.48	330	41	12.4	79	7	8.8	
20.2.48	213	14	6.6	149	13	8.7	
21.2.48	140	17	12.1	225	21	9.3	
22.2.48	228	17	7.5	108	8	7.4	
23.2.48	172	14	8.1	188	10	5.3	
26.2.48	253	28	11.0	135	31	22.9	
27.2.48	167	39	23.4	107	19	17.8	
28.2.48	114	24	21.1	84	16	19.0	
1.3.48	214	30	14.0	125	22	17.6	
2.3.48	223	26	11.6	84	5	6.0	Sig.
8.3.48	243	26	10.7	131	37	28.0	
7.3.48	190	29	15.3	99	8	8.0	
8.3.48	297	24	8.1	147	12	8.2	
9.3.48	186	21	11.3	201	23	11.4	
12.3.48	170	20	11.8	181	28	15.5	Sig.
13.3.48	142	17	12.0	140	32	22.9	
14.3.48	194	26	13.4	72	18	25.0	
15.3.48	150	29	19.3	117	26	22.2	
16.3.48	186	29	15.6	158	16	10.1	
19.3.48	207	46	22.2	178	16	9.0	Not sig.
20.3.48	247	22	9.0	304	30	9.9	
21.3.48	403	26	6.5	174	28	16.1	
22.3.48	217	26	12.0	102	24	23.5	
23.3.48	322	42	13.1	191	20	10.5	
Total	8,776	730	8.3	3,541	477	13.5	Not sig.

#### CONCLUSION.

The results of the work described in this paper show that DDT sprayed on cattle or attracting screens can be used to kill a high percentage of flies that may alight on them. Much work remains to be done, and this has been carried on by my colleagues since I left Tanganyika in 1945.

## SUMMARY

(1) The paper describes a method for exterminating tsetse-flies by "flooding" isolated tsetse-infested areas with DDT-treated cattle

(2) Introduction explains the reasons mainly economical, why this particular method of applying DDT has been attempted, instead of more obvious methods of application

(3) Various simple laboratory tests demonstrating the toxicity of DDT to various species of tsetse are described

(4) DDT was shown to be lethal to the following species of tsetse: *G. palpalis fuscipes* from Uganda, *G. palpalis martinii* from Abercorn, Northern Rhodesia, *G. morsitans morsitans* from Kondoa, Tanganyika, *G. morsitans orientalis* from Kingolwara, Tanganyika, *G. pallidipes*, *G. severrertoni* from Shinyanga, Tanganyika, *G. austeni* from Kingolwara, Tanganyika, *G. fuscipleuris* and *G. brevipalpis* from Kisumu, Kenya, and *G. longipennis* from Mbulu, Tanganyika. Later it has also been shown that DDT is lethal to *G. palpalis palpalis*, *G. tachinoides* and *G. morsitans submorsitans* from Kaduna, Nigeria

(5) Conclusions reached from a series of experiments with various emulsions containing DDT were that numerous small residual crystals were more effective than a few large crystals although the total weight of DDT per unit area was the same in both examples. However, the large crystals are effective for a longer period than the small ones

(6) A description of the method of testing the effectiveness of DDT preparations in the field is given

(7) Tables show the comparative number of marked recaptures from treated and untreated (control) oxen, also the same preparation on screens. The preparations retained their effectiveness longer on screens presumably since they were subjected to less wear and tear than on the cattle

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## APPENDIX

FORMULAE OF THE DDT PREPARATIONS USED IN THE EXPERIMENTS DESCRIBED IN THE PAPER.

- K/1 5 grammes of DDT (active isomer varying with each batch of DDT). 100 grammes of commercial kerosene. Dissolved using mechanical stirrer.
- CT/2 45 grammes of DDT dissolved in 100 grammes of carbon tetrachloride.
- Gh/5 100 parts of formula K/1 emulsified with 5 grammes of local gum arabic dissolved in 100 c.c. of water with 0.1 gramme of sodium arsenite as preservative.
- Bh/4 100 parts of formula K/1 emulsified with 100 parts of ox serum and 0.1 gramme of sodium arsenite as preservative. The ox serum was prepared by allowing the freshly drawn blood to clot and then separate from the serum.
- Lh/3 100 parts of formula K/1 emulsified with 5 grammes of gelatine dissolved in 100 c.c. of water with 0.1 gramme of sodium arsenite as preservative.
- GK/3A }  
SK/4A } As for other formulae but only  $\frac{1}{2}$  gramme of DDT dissolved in 100 grammes  
Lh/3A } of kerosene.
- GD/10 9 grammes of DDT dissolved in 100 c.c. of Diesel fuel oil using mechanical stirrer. This was emulsified with 5 grammes of local gum dissolved in 100 c.c. of water with 0.1 gramme of sodium arsenite as preservative.
- D/11 9 grammes of DDT dissolved in 100 c.c. of Diesel fuel oil.
- SD/12 9 grammes of DDT dissolved in 100 c.c. of Diesel fuel oil emulsified with 100 c.c. of serum.

Various proportions of continuous phase to disperse phase were made up and tested out in different laboratory experiments. Mixtures of DDT with paraffin wax, ground-rot oil, and with resin were also tried. It was found that unless fairly high percentages (30 to 100 per cent.) of DDT were used that these substances masked the effects of the DDT.

Unfortunately it was not possible to measure and record the various specifications of the kerosenes and Diesel oils used. These varied with different batches of emulsions.

# SIDELIGHTS ON MALARIA IN MAN OBTAINED BY SUBINOCULATION EXPERIMENTS

BY

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Medical Services, Australian Military Forces, for permission to publish this paper

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## INTRODUCTION

This communication is chiefly concerned with the value of subinoculation in determining the biological activity and distribution of plasmodia in man, the particular stage in the life cycle of the parasite on which anti malaria drugs act, and indirect evidence of the existence of a human exoerythrocytic cycle.

Few authorities today would subscribe to the long-held view of SCHAUDINN (1902) that sporozoites directly penetrate the red cells soon after inoculation by infected anopheline vectors

Until exoerythrocytic or *ee* forms have been irrefutably demonstrated in human malaria, their existence no doubt will be subject to controversy. Despite the failure to demonstrate *ee* forms in man it is necessary to postulate their existence as no other hypothesis will satisfactorily explain the results obtained by subinoculation experiments and the different response to treatment of sporozoite and trophozoite-transmitted malaria in *Plasmodium vivax* and *P. falciparum* infections

In avian malaria, there has existed for some years abundant evidence of the existence of non-pigmented parasites in endothelial and reticulo-endothelial cells at a time when asexual parasites are present in blood films. RAFFAELE (1936) described such forms in canaries infected with *P. relictum*, KIKUTH and MUDROW (1937) in canaries infected with *P. cathemerum* and JAMES and TATE (1937) in canaries infected with *P. gallinaceum*, it was to this cycle that JAMES and TATE (1937) first applied the term exoerythrocytic or *ee* forms

These results were fully confirmed and extended, but several years elapsed before a pre-erythrocytic cycle intervening between the inoculation of sporozoites and the appearance of asexual parasites in the red cells was experimentally demonstrated in avian malaria

REICHNOW and MUDROW (1943), and MUDROW and REICHNOW (1944) investigated the exoerythrocytic cycle by injecting sporozoites of *P. praecox* into the tissues of canaries. Most of the injected sporozoites were destroyed *in situ* but a few entered cells of the reticulo-endothelium, here, after resting for 4 hours, the sporozoites increased in size and later nuclear division commenced. Generally six divisions were observed before segmentation into merozoites occurred, the cycle lasting 36 hours. These merozoites subsequently invaded fresh reticulo-endothelial cells and again produced schizonts and merozoites. In the third cycle similar development occurred, but in some schizonts the nuclear division proceeded further to produce 128 or more merozoites instead of sixty-four merozoites. The schizonts with the larger number of nuclei (microschizonts) produced smaller merozoites containing small nuclei and little cytoplasm (micromerozoites). The micromerozoites later escaped into the blood, invaded erythrocytes and commenced the asexual cycle. The schizonts with the smaller number of nuclei (macroshizonts) gave rise to macromerozoites which only entered reticulo-endothelial cells and never invaded the red blood corpuscles

As a rule the first micromerozoites to invade red cells were found at the end of the third endothelial cycle, but occasionally a few were produced in the second cycle. By the fifth cycle practically all the schizonts were microschizonts and all the merozoites were micromerozoites. Approximately 10 per cent of the micromerozoites failed to form schizonts, they developed in



gametocytes and their sex differentiation was clearly established before they had entered the red blood corpuscles.

HUFF and COULSTON (1944), by the inoculation of great numbers of sporozoites (*P. gallinaceum*) into localized skin areas in chicks demonstrated in detail the schizogonous cycle in the reticulo-endothelial cells. Within half an hour inoculated sporozoites were shown to penetrate the cells of the reticulo-endothelial system. The term cryptozoites was suggested for exoerythrocytic parasites of the first cycle and the term metacryptozoites for those of the second and subsequent cycles. Not more than two generations preceded the first appearance of parasites in the red cells in *P. gallinaceum*. More recently HUFF and COULSTON (1946) introduced the term phanerozoites for those exoerythrocytic parasites which persisted in the reticulo-endothelium and endothelial cells in *P. gallinaceum*.

DAVEY (1944) suggested there may be primary and secondary "tissue phase" parasites, but later (1946) adopted the classification of primary and secondary exoerythrocytic forms, the primary being those which develop directly from the sporozoite. The secondary exoerythrocytic forms are the older forms and are usually co-existent in the avian host with blood parasites. In birds the secondary exoerythrocytic forms may originate directly from the primary exoerythrocytic forms or indirectly from asexual blood parasites. The latter contention is borne out by the work of COULSTON and MAXWELL (1941), who produced experimental infections with *P. circumflexum* by the injection of a single parasitized corpuscle. As will be shown later the available evidence does not support the view that e.e. forms in man can arise from asexual parasites.

In the present communication the term pre-erythrocytic forms refers to early e.e. forms which are believed to occur in man in the first three or four schizogonous cycles in reticulo-endothelial cells before blood forms appear i.e. the cryptozoites and metacryptozoites of HUFF and COULSTON (1944). The term late e.e. forms is used for those e.e. forms which are believed to persist in reticulo-endothelium or endothelial cells—they correspond to the phanerozoites of HUFF and COULSTON (1946) or the persistent macromerozoites of REICHENOW and MUDROW (1943).

FAIRLEY and his colleagues (1945) recorded indirect evidence that these late e.e. forms persist in vivax infections but not in falciparum malaria in man.

#### TECHNIQUE OF SUBINOCULATION

Throughout this paper the term subinoculation refers to the transfer of whole blood from a volunteer who had been exposed to experimental malaria infection to a volunteer who had never previously been exposed to infection with the species of plasmodium used in the experiment and who, in consequence, had been afforded no opportunity of developing premunity. Previous workers



TABLE I.

SPOROZOITIEMES PERFORMED DURING THE FIRST HOUR AFTER RESPONSE OF THE DONORS  
TO INFECTIVE MOSQUITOES.

(P. SWER.)

Donor				Subinoculation			Recipient
Number and Name of volunteer	Number of infective bites	Duration of biting (in minutes)	First parasites in thick blood films (days after infection)	Minutes after biting ceased	Volume of blood injected (c.c.)	Result	First parasites in thick blood films (days after receiving blood)
1. Re.	12	8	12	During biting	800	+	17
2. Wh.	12	8	10	7	800	+	12
3. Mog.	11	8	12	10	200	0	0
4. Moa.	12	6	12	30	200	+	26
5. Hal.	14	9	11	30	200	?	0
6. Bol.	17	10	12	60	800	0	0
7. Qua.	21	17	12	60	800	0	0

+ = positive. 0 = negative. ? = doubtful.

These results indicate that in sporozoite-induced vivax malaria viable sporozoites may gain access to the blood during the period of biting and be present up to at least 30 minutes after cessation of biting.

Distinct differences have been noted between the course of the vivax infection in the donors who received sporozoites by mosquito inoculation and the recipients who received sporozoites by blood transfusion from the donors. These differences are probably related to the decreased dosage of sporozoites received by the recipients, and are summarized in Table II.

#### (a) Primary attacks.

Relevant data are given in Table II. The chief difference noted between the recipients and donors are graphically depicted in Charts 1, 2 and 3 (p. 628) and may be summarized as follows —

1. The longer period manifest in the recipients between exposure to infection and the appearance of the first demonstrable parasites in thick blood films. The differences observed were 5, 3 and 24 days respectively.
2. The slower rate of increase in parasite densities.



VIVAX MALARIA. Parasitological differences between donors bitten by infective mosquitoes, and the recipients of their blood containing sporozoites. Recipients 1 and 2 received 500 c.c. of blood; Recipient 4 200 c.c.

CHART 1  
SUBINOCULATION DURING EXPOSURE

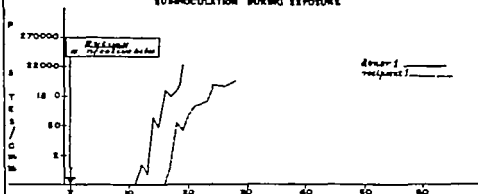


CHART 2  
SUBINOCULATION 7 DAYS AFTER EXPOSURE

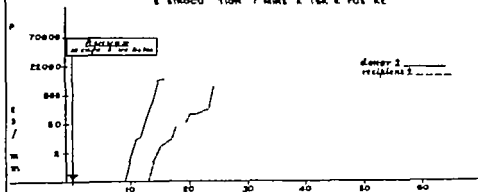


CHART 3  
SUBINOCULATION 30 DAYS AFTER EXPOSURE



Days after infection.

at this early stage was undertaken for reasons not related to the medical urgency of the case

(b) *Secondary attacks*

The three recipients showed less liability to relapse, i.e., longer periods of freedom and fewer secondary attacks in the period of observation. Donors and recipients were given exactly the same anti-malaria treatment which consisted of the routine Q A P course\* with or without atebryn maintenance.

One recipient (No 2) of 500 c.c. of blood had a secondary attack of vivax malaria after ceasing a standard course of treatment without atebryn maintenance. This attack occurred 291 days after ceasing therapy for his primary attack. Neither of the other two recipients, who developed primary malaria, had developed a secondary attack. In contrast to this, all the donors had secondary attacks. One relapsed on the 49th, 156th and 256th day, the other two relapsed on the 85th and 119th day respectively.

(c) *Atypical clinical syndrome associated with blood transfusion of sporozoites*  
(P vivax)

Perhaps the most interesting case was that of the recipient (Bun) who received 500 c.c. of blood 30 minutes after exposure of the donor (Hal) to infective bites (Table I), he subsequently developed splenomegaly, hepatomegaly, some degree of leucopenia and occasional fever to 100° F (Chart 4). In spite of frequent examination of 1 to 2 c.mm. of blood in thick blood films, no parasites were ever demonstrated. Neither injections of adrenalin and insulin nor chilling (1 hour at -10° C) produced clinical or parasitological evidence of active malaria. Subinoculation of 800 c.c. whole blood on the 67th day after the initial exposure was negative, the recipient failing to develop demonstrable blood parasites or symptoms of malaria.

It is unfortunate that subinoculation was not done earlier in this case, i.e., between the 35th and 45th days (see Chart 4) for at this period there was suggestive clinical evidence of malaria, and it is not unreasonable to suppose it may have been associated with a submicroscopic density of parasites which subinoculation might at that time have revealed even though careful microscopic examination of the blood failed to do so. At other times the clinical features may possibly have been related to the activities of an exoerythrocytic vivax cycle which will be commented on later.

Thick films (1 c.mm.) were examined daily from the day of receiving the inoculum to the 30th day thereafter with entirely negative results. Up to this time there was little, if any, evidence that malaria infection had resulted,

\* Q A P course. The standard course consisted of quinine sulphate (grains 30 daily) for 3 days, atebryn in dosage of 0.6, 0.5, 0.4, 0.3 and 0.2 gramme for the next 5 days, and quinine (grains 15) and plasmoquine base (0.03 gramme) daily for 5 days. Atebryn maintenance consisted of 0.1 gramme daily for 42 days.

and the blood was not examined from the 31st to the 34th day. On the 35th day however there was fever and splenomegaly and re-examination of the blood commenced. Daily observations were made from the 35th to the 62nd days, and subsequently three times a week until the 120th day—again with entirely negative results. Then he was discharged from the Medical Research Unit. For another 204 days he remained on the mainland of Australia. Throughout this period he developed no overt malaria attacks, and remained perfectly fit. Unfortunately some 314 days after original exposure to infection this patient went to a hyperendemic area of malaria in New Guinea where he contracted falciparum malaria. After an interval of 324 days *i.e.* 638 days after the original experimental infection, he was brought back to Cairns for re-investigation, and here he developed vivax malaria after ceasing to take suppressive atabrin. It is highly probable that this attack of vivax malaria was a re-infection acquired in New Guinea, but it may have been a late relapse following the original experimental infection at Cairns.

The case is so unique in our experience that it is recorded in detail below (Chart 4).

*Infection.*—This volunteer Bun. received 500 c.c. of whole blood by direct transfusion from donor Hal; transfusion lasted 4 minutes, and commenced 30 minutes after the last mosquito engorged on the donor.

*Donor.*—Hal. was bitten by fourteen *A. punctulatus punctulatus* infected with viable *P. vivax* sporozoites. The sporozoites age was 6 days, salivary glands were heavily infected, and the batches used were 87 per cent. infective.

*Course.*—During the first fortnight after receiving the inoculum the recipient, volunteer Bun., had some tenderness of his spleen and liver but no temperature reaching 99° F or more was recorded. He complained of no symptoms. No significant changes were noted in white cell counts, red cell counts, or in haemoglobin values. Parasites were not demonstrated.

During the second fortnight, no symptoms, signs, or haematological evidence of any abnormality were recorded. Daily blood films were negative even though as much as 1 to 2 mm. were examined in thick films.

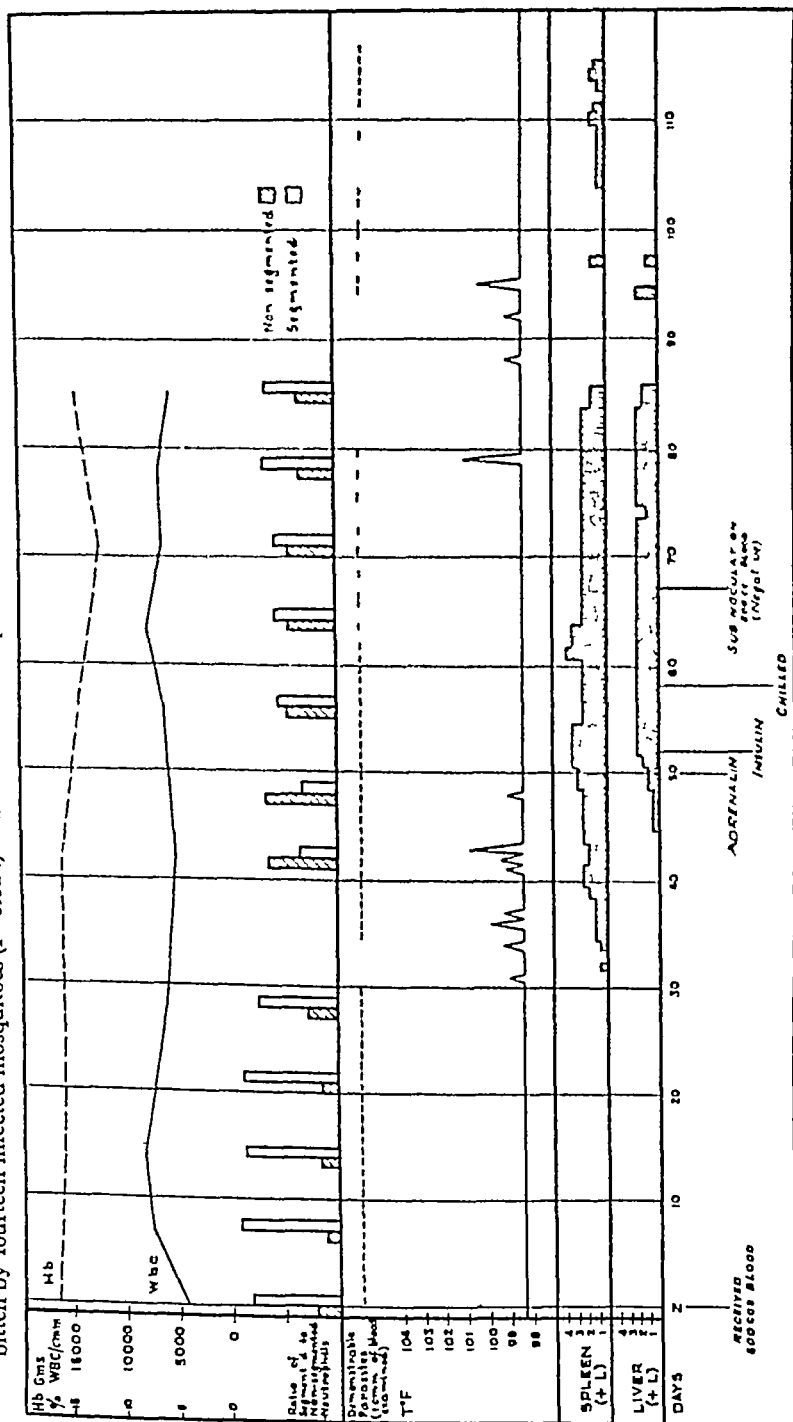
On the 31st day after receiving his inoculum he complained of headache, and between the 31st and 39th days had frequent headaches, malaise, some anorexia and rise of temperature on one occasion to 99° F and on another occasion to 100.2° F. His spleen was first palpable on the 31st day and by the 39th day was slightly more than one finger's breadth below the left costal margin at the end of deep inspiration. The liver was not palpable or tender at this time. There was definite and marked left shift of polymorphonuclear leucocytes which is a common finding at certain stages in malaria infection. No parasites were demonstrated in thick blood films though 1 c.mm. was examined daily.

Between the 43rd and 63rd days there was an occasional temperature of 99° F and on the 79th day of 101° F. Symptoms were minimal and restricted to an occasional headache. The spleen was palpable to two fingers breadth during this period except between the 51st and 54th days and 61st and 63rd days when it was palpable and firm to three fingers breadth below the left costal margin at the end of deep inspiration.

The liver was first definitely palpable on the 45th day and remained palpable during this period of time, *i.e.*, to the 83rd day extending to two fingers breadth below the right subcostal margin between the 57th and 63rd days. At no time was there any rash, adenitis or any other symptom or sign suggesting any intercurrent infection. The total white cell count remained relatively constant and the left shift of the polymorphonuclear leucocytes persisted throughout this period, showing but minor variations. There was some fall in haemoglobin—the initial value was 16.3 grammes per 100 c.c., on the 42nd

CHART 4

Atypical clinical syndrome in a recipient following a direct transfusion of 500 c.c. of blood made 30 minutes after the donor had been bitten by fourteen infected mosquitoes (*P. vivax*). The donor developed overt malaria and parasites 11 days after exposure





day it was 16.0 grammes per 100 c.c., and by the 71st day it had fallen to 12.3 grammes per 100 c.c.

In an attempt to induce an attack of malaria 0.5 c.c. of adrenalin (1/1000 solution) was given each hour for 4 hours on the 60th day. This made the volunteer shaky and distressed, but no parasites were demonstrated nor was pyrexia produced. On the 62nd day insulin was given and the blood sugar reduced to 80 mg. per 100 c.c. without effect on his general condition, and again parasites were not demonstrated.

On the 68th day the volunteer was in refrigeration chamber at temperature of  $-10^{\circ}\text{C.}$ , clad only in trousers and boots, and stayed there voluntarily for 1 hour with minimal movement. No fever or other evidence, either clinical or pathological, of active malaria resulted from this chilling.

On the 67th day 800 c.c. whole blood were transfused into another volunteer the transfusion occupying 6 minutes. This acute loss of blood did not produce any evidence of malaria in the donor Burn, and the recipient showed no evidence of having received any malaria parasites as result of subinoculation with this large volume of blood.

Between the 63rd and 124th days an occasional rise of temperature to  $99^{\circ}\text{F.}$  and on one occasion rise to  $100.4^{\circ}\text{F.}$  were recorded. The spleen and liver gradually became smaller and finally were impalpable. Symptoms disappeared and there was an improvement in haemoglobin value which reached 14.5 grammes per 100 c.c. on the 85th day. The polymorphonuclear left shift disappeared and the volunteer was discharged from the experiment apparently well.

During the subsequent 200 days the volunteer performed the ordinary duties of his unit and was not admitted to medical unit on any occasion (total period of 324 days since receiving the original inoculum). Subsequently he went to New Guinea.

H had one attack of falciparum malaria for which he received the standard course of treatment. He continued with suppressive atabrin, 0.1 grammes per day and remained well. H was re-admitted to the Medical Research Unit at Cairns and ceased taking atabrin. Re-admission was 633 days after the original experiment; he had spent 314 days in New Guinea.

On admission his skin showed slight atabrin staining. His spleen and liver were not palpable. On the 4th and 8th days after admission there was tenderness over the liver and this sign was elicited again on the 11th and 12th days. Nineteen days after ceasing atabrin suppression his temperature rose to  $98^{\circ}\text{F.}$  and he had feeling of fullness in the head. Next day the temperature rose to  $101.6^{\circ}\text{F.}$  and he sweated as it fell. On the following day—the 21st since cessation of atabrin—trophozoites of *P. vivax* were found in thick film. On this day his spleen became palpable and it increased in size until the 8th day after the first appearance of parasites when it was slightly more than two fingers breadth below the costal margin. The liver became tender again but was not palpably enlarged. The temperature followed tertian course and treatment was commenced on the 8th day after the first appearance of parasites.

A course of paludrine, 1.0 gramme per day for 14 days, was given. The spleen subsided but was just palpable at the costal margin when he was discharged at the end of the course of treatment.

#### (d) Comment.

(1) No previous observations on subinoculation at the time of biting or shortly afterwards appear to have been made in man, but BOYD and STRATHMAN-THOMAS (1934) showed experimentally that excision of the bitten area a few moments after infective mosquitoes had engorged did not prevent infection. They concluded that in such cases sporozoites were injected directly into the blood stream. They also found that when infective mosquitoes were applied to the skin of a blister raised by the application of cantharides, malaria infection followed. They concluded that sporozoites inoculated into the tissues could traverse the tissues and gain access to the circulating blood. Our observations

on subinoculation support the view that sporozoites may be inoculated directly into blood vessel by infective mosquito or subsequently gain access to the circulation after being inoculated into skin and subcutaneous tissues. The irregular results of subinoculation over the first 60 minutes obtained in these experiments is explicable in terms of the variable time taken by mosquito-inoculated sporozoites in traversing the tissue and wall of the blood vessels and gaining access to the circulation, and the variable time taken to remove them from circulating blood by the reticulo-endothelium.

(2) BOYD (1940) concluded that the dose of sporozoites in *vivax* malaria exert a significant influence on the subsequent infection. Thus the duration of the clinical attack appeared to vary directly with the dose of sporozoites and inversely with the inoculation period, while the relapses were probably more severe and more frequent after bites with heavily infected mosquitoes than with lightly infected mosquitoes. SMITH (1946), as a result of recent experiments, suggests that about 2000 sporozoites are necessary to ensure a normal incubation period and that true latency occurs only when the sporozoites injected are too few to set up a primary attack.

Data obtained from experimentally infected volunteers at Cairn, indicated that no modifications of the primary or secondary attacks resulted in *vivax* malaria which were dependent on the smallness of dose of sporozoite inoculated provided more than two mosquitoes with heavy sporozoite infection of the salivary glands had enzorged.

In the subinoculation experiments performed during the first hour after exposure of the donor to bite of mosquitoes infected with *P. vivax*, definite differences between donors and recipients were, however, noted both in the primary attack and in the tendency to secondary attack.

If it be assumed that no significant modification or developmental changes have occurred in the short period of time the sporozoites were circulating in the blood of the donor and the recipient before assuming an intracellular location, then the observations on the donors and recipient support the view that the dose of sporozoite may influence the primary attack and the subsequent liability to have secondary attacks. It is certain that the recipients had a very much smaller dose of sporozoites than the donors for the latter received eleven to twenty-one infective bites from mosquitoes with medium to heavy sporozoite infection of the salivary gland. On the other hand, the recipient received one-tenth to one-twenty-fifth of the total blood volume of the donor transfused in a few minutes. The actual dosage received would vary with the number of sporozoites in the donor's blood at the commencement of the transfusion, the number of fresh sporozoites gaining access to the circulation from the subcutaneous tissues and the number removed from the circulating blood by the reticulo-endothelium during the transfusion.

(3) The atypical clinical syndrome described in the recipient (Bun) may also be related to small sporozoite dosage, but here it is possible an additional

factor related to schizogony of the e.c. forms may be involved. If e.c. forms in *P. vivax* correspond to those observed by REICHNOW and MUDROW (1943) in *P. praecox* in canaries the hypertrophy of reticulo-endothelium with splenomegaly and hepatomegaly unassociated with overt attacks and parasites in the blood is explicable on the assumption that exoerythrocytic schizogony did not proceed beyond the production of macroschizonts and macromerozoites which are confined to reticulo-endothelium, or if micromerozoites were produced their number and vitality were so reduced that they never attained a density demonstrable by the microscope. Failure to produce viable micromerozoites would account for the absence of asexual forms in the red blood corpuscles and overt attacks of malaria, while progressive involvement of the reticulo-endothelium by macromerozoites would explain the splenomegaly and hepatomegaly. If so such material should prove ideal for studying the exoerythrocytic cycle. A more prolonged sojourn of transfused sporozoites in the circulating blood of two instead of one individual might occasionally lead to curtailed development within reticulo-endothelial cells along the lines indicated.

The case history is undoubtedly one of considerable interest since similar atypical clinical syndromes which have hitherto remained unrecognized may be occurring in naturally acquired infections under conditions not yet adequately studied.

In nature *vivax* malaria infection with minimal sporozoite dosage is most likely to occur at the end of the malaria season when the anopheline population decreases and the density of sporozoite infections of the salivary glands tends to be minimal. Spontaneous suppression of the primary fever and the postponement of the overt attack for periods of 6 to 9 months are recognized to occur in Europe and elsewhere under such circumstances. Here the age of the sporozoite as well as minimal dosage may be a responsible factor.

Other observations at Cairns suggest that an increase in the sporozoite age or a decrease in the number of infective bites to two or less produces a similar result—namely fewer inoculated viable sporozoites.

## (2) SUBINOCULATIONS PERFORMED DURING THE FIRST 11 DAYS AFTER EXPOSURE OF THE DONORS TO INFECTIVE MOSQUITOES (*P. vivax*).

Two groups of donors were investigated from this viewpoint. The first, Group A, received no drug treatment until after overt malaria had developed the second, Group B were taking daily doses of atabrin or various suppressive drugs with a similar schizonticidal action, i.e. quinine, sontochin and resochin. Resochin (S.N.7818) is now called chloroquine or aralen.

### (a) Group A—Controls

The donors in Group A were exposed to biting from two to 101 *A. punctulatus punctulatus* infected with viable sporozoites of New Guinea strains of *P. vivax* at single sessions lasting 10 to 20 minutes. Two individuals received two infective bites, twelve received from ten to nineteen infective bites, while one volunteer actually received 101 infective bites at the one session.

The batches of mosquitoes were from 58 to 100 per cent. infective (average 90 per cent), the gland infections were medium-heavy to heavy except in one batch in which they were light, and the sporozoite age was 2 to 14 days (average 7 days)

The detailed results in this Group A are given in the accompanying Table III. It will be noted that eighteen out of eighteen recipients received

TABLE III

GROUP A CONTROLS \* SUBINOCULATIONS PERFORMED DURING THE FIRST 11 DAYS AFTER EXPOSURE OF THE DONORS TO INFECTIVE MOSQUITOES (*P vivax*)  
(SINGLE EXPOSURE ON DAY 0)

Donor			Subinoculation			Recipient
Name	Number of infective bites	First parasites in thick blood films (days after infection)	Time after biting ceased Days/hrs	Volume in c.c	Result Positive (+) Negative (O)	First parasites in thick blood films (days after receiving blood)
Whi	12	10	1	500	O	0
Hai	14	11	2	500	O	0
Hea	12	12	3	500	O	0
Hea	12	12	4	500	O	0
Ric	13	12	5	500	O	0
Dav	10	12	5/16	200	O	0
But	10	12	5/16	200	O	0
Hem.	19	14	6	200	O	0
Vos	16	12	6	200	O	0
Tol	10	12	6/14	200	O	0
Wic	11	12	6/14	200	O	0
McC	101	10	6/14	200	O	0
Hem	19	14	7	200	O	0
Vos	16	12	7	200	O	0
McC	101	10	7/14	200	O	0
But	10	12	7/15	200	O	0
Dav	10	12	7/15	200	O	0
Jon	17	9	8	500	O	0
Ben	2	12	8/14	200	+	12
Tol	10	12	8/19	200	+	8
Wic	11	12	8/19	200	+	12
Moa	12	12	8/23	200	+	6
Mog	11	12	9/23	200	+	7
Mof	2	11	10/14	200	+	10

\* No treatment was given to any of these volunteers until overt malaria had developed

200 to 500 c.c. of blood collected 1 to 8 days after exposure of the donors to ten to 101 infective bites (*P. vivax*) all the recipients failed to develop vivax malaria. On the other hand, six out of six recipients of 200 c.c. of blood, collected 208 to 254 hours after exposure to two to twelve infective bites (*P. vivax*) developed demonstrable parasites in thick films some 6 to 12 days after the transfusion.

(b) *Group B—Receiving suppressive schizonticidal drugs*

These volunteers were bitten by two to fifty *A. punctulatus punctulatus* with sporozoites of *P. vivax* (New Guinea strains) in their salivary glands. The batches were from 60 to 100 per cent. infective the gland infections varied from light to very heavy and the sporozoite age was from 2 to 20 days.

The detailed results in Group B are given in the accompanying Table IV. It will be noted that five out of five recipients of 200 c.c. of blood collected from donors some 182 to 183 hours after exposure to twenty to twenty-two infective bites failed to develop vivax malaria. On the other hand, twenty two out of twenty-two recipients of 20 to 200 c.c. of blood, collected 208 to 211 hours after the donors had been exposed to from two to fifty infective bites, developed vivax malaria parasites in thick films some 6 to 17 days later. This occurred despite the fact that the donors were taking daily doses of anti malaria drugs adequate for suppressive purposes.

(c) *Combined results*

The results of subinoculations obtained in these two groups, Group A and Group B and detailed in Table III and Table IV respectively have been condensed in Table V (p. 638).

It will be seen from a perusal of Table V that the blood of twenty-three out of twenty three volunteers collected 1 to 8 days after exposure to sporozoite infection uniformly failed to transmit malaria to the recipients, while the blood of twenty-eight infected volunteers collected from 8½ to 10½ days after exposure to infection, invariably produced overt malaria in the recipients. Uniform results were obtained in the two groups, A and B and it proved immaterial whether or not the volunteers were taking suppressive drugs of schizonticidal type over the period under consideration.

The earliest positive subinoculations occurred on the 9th day—some 206 hours after exposure. The latest negative subinoculation was obtained on the 8th day—182 hours after exposure. Thus it appears that the first generation of erythrocytic parasites of *P. vivax* (micromerozoites) were first released into the circulation between 182 and 206 hours (i.e. 8½ days) after inoculation of sporozoites by infective mosquitoes (Chart 5). The original sporozoites after gaining access to the circulation appear to be rapidly removed—probably by the reticulo-endothelium. analogy with avian malaria suggests they undergo

TABLE IV

TABLE IV. SUMMARY OF THE RESULTS OF THE TREATMENT OF 100 PATIENTS WITH MALARIA IN THE TROPICS. THE RESULTS ARE GIVEN IN THE FOLLOWING TABLE. THE RESULTS ARE GIVEN IN THE FOLLOWING TABLE. THE RESULTS ARE GIVEN IN THE FOLLOWING TABLE.

Name	Dose	No. of patients	Dose				No. of patients	No. of patients
			1st	2nd	3rd	4th		
C	100 mg	21	0	4	7	14	0	0
A	100 mg	21	0	4	7	14	0	0
D	100 mg	21	0	4	7	14	0	0
L	100 mg	21	0	4	7	14	0	0
D	100 mg	21	0	4	7	14	0	0
A	100 mg	22	0	4	7	14	0	0
S	100 mg	22	0	4	7	14	0	0
V	100 mg	22	0	4	7	14	0	0
M	100 mg	22	0	4	7	14	0	0
R	100 mg	22	0	4	7	14	0	0
S	100 mg	22	0	4	7	14	0	0
G	100 mg	21	0	4	7	14	0	0
C	100 mg	21	0	4	7	14	0	0
F	100 mg	21	0	4	7	14	0	0
T	100 mg	21	0	4	7	14	0	0
K	100 mg	10	0	4	7	14	0	0
C	100 mg	10	0	4	7	14	0	0
J	100 mg	2	0	4	7	14	0	0
H	100 mg	10	0	4	7	14	0	0
T	100 mg	2	0	4	7	14	0	0
B	100 mg	2	0	4	7	14	0	0
G	100 mg	10	0	4	7	14	0	0
M	100 mg	10	0	4	7	14	0	0
F	100 mg	20	0	4	7	14	0	0
C	100 mg	22	0	4	7	14	0	0
R	100 mg	20	0	4	7	14	0	0
M	100 mg	20	0	4	7	14	0	0

\* Retochin (S N 7015) is now called chloroquine or aralen in U.S.A.

TABLE V

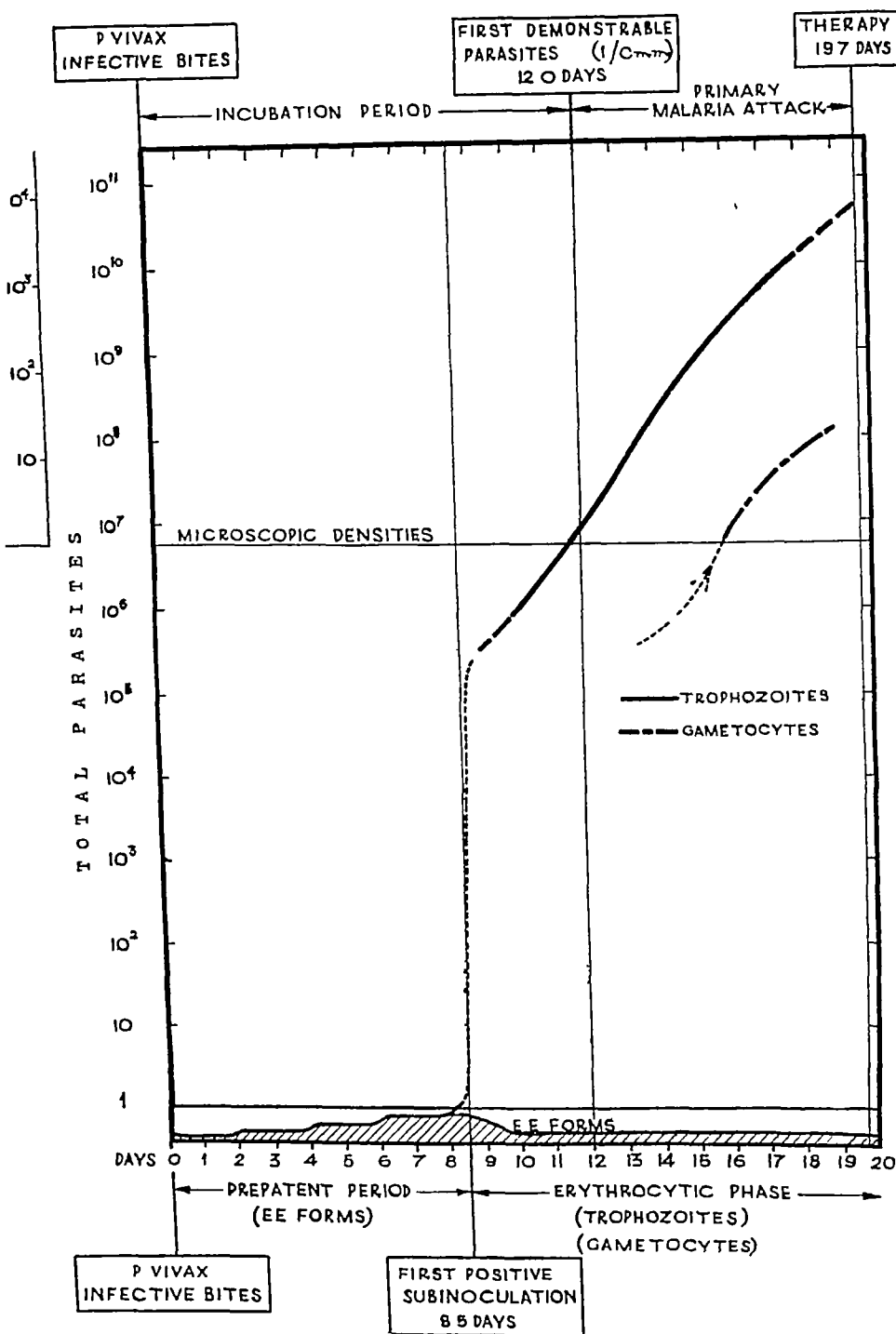
RESULTS OF FIFTY-ONE SUBINOCULATIONS PERFORMED BETWEEN THE 1ST AND 11TH DAYS AFTER EXPOSURE OF VOLUNTEERS TO MOSQUITOES INFECTIVE WITH *P. vivax*.

(GROUPS A AND B.)

Days since exposure of donor	Group A.			Group B.		
	Number of subinoculations.	Results.		Number of subinoculations.	Results.	
		Positive.	Negative.		Positive.	Negative.
1	1	0	1	—	—	—
2	1	0	1	—	—	—
3	1	0	1	—	—	—
4	1	0	1	—	—	—
5	1	0	1	—	—	—
8½	4	0	4	—	—	—
9½	5	0	5	—	—	—
7½	3	0	3	5	0	5
8	1	0	1	—	—	—
8½	4	4	0	10	10	0
9½	1	1	0	12	12	0
10½	1	1	0	—	—	—
Total	24	6	18	27	22	5

an intracellular schizogamous cycle with the production of cryptozoites and metacryptozoites. If the pre-erythrocytic schizogamous cycle lasts approximately 48 hours (as in the asexual erythrocytic cycle), the findings suggest that there would be four complete cycles within the endothelial cells before the first erythrocytic parasites (macromerozoites) were liberated into the blood stream.

One volunteer (Jon.) who was having no suppressive drugs, received seventeen infected bites from batches of *A. punctulatus punctulatus* which were 40 to 100 per cent. infective with sporozoites 2 to 5 days old. Subinoculation of 500 c.c. of his blood 192 hours (8 days) after exposure to infection was negative, but a single trophozoite (*P. vivax*) was seen in a thick blood film collected 24 hours later i.e. 216 hours after exposure to infection. These observations suggest that a relatively large number of erythrocytic parasites were liberated from the pre-erythrocytic or early c.c. forms some 8½ days after inoculation of sporozoites. It is noteworthy that though the number of infective bites given to the donors varied from two to 101 the dosage of sporozoites did not influence subinoculation results.



Note increase in density of pre-erythrocytic or hypothetical early e e forms with liberation of erythrocytic parasites (micromerozoites) after 8 days. Subsequently there is a relatively slow increase in parasitic density, the curve being sigmoid in shape. In contrast to what happens in falciparum malaria, late e e forms are regarded as persisting.



Parasites of *P. vivax* were first demonstrated microscopically in thick films to the order of 1 per c.mm. on an average of 12 days after exposure, the range being 10 to 17 days. Trophozoite densities were observed gradually to increase in the majority of volunteers till a density of 2,580 to 24,000 per c.mm. (mean of 8,270 per c.mm.) was reached on the day of first therapy. The curve of increasing parasite densities was sigmoid in form (Chart 5) showing a relatively slow initial and final rate of increase with the most rapid rate between the 12th and 14th day. By the 19th or 20th day therapy was usually required, the average being 19.7 days after exposure (limits 15 and 22 days). By this time the volunteers generally had symptoms of such a degree of severity that it was considered undesirable to withhold therapy further. When treatment was withheld and the vivax infection was allowed to run its natural course in the experimentally infected volunteer primary fever persisted for several weeks before spontaneous remission ensued. With the establishment of premunity febrile attacks ceased but at times parasites appeared in the blood and were readily demonstrable microscopically and unassociated with major clinical features of malaria.

(d) *Nature of malaria attacks in recipients of subinoculation*

The positive recipients all developed typical attacks of trophozoite-induced vivax malaria. Malaria parasites were first demonstrated in the recipients between the 6th and 12th days after receiving their inocula. This indicated that between three and six schizogones occurred before sufficient parasites were present to be demonstrated in thick blood films when 1 c.mm. is examined daily.

No significant difference between the behaviour of recipients receiving their inocula early (i.e., 8½ days) rather than late (i.e. 10½ days) has been observed. As a rule trophozoite-induced vivax malaria is radically cured by one course of treatment. However one recipient injected with blood from Donor 4 (No.), collected on the 9th day after exposure (215 hours), developed a secondary attack 26 days after ceasing a course of quinine sulphate (grammes 2.0) and plasmoquine base (gramme 0.03) daily for 10 days.

Secondary attacks have not been observed in the other five positive recipients of Group A. If it is characteristic of recipients of blood containing first generation erythrocytic parasites (*P. vivax*), i.e., micromerozoites to have secondary attacks after treatment, they would differ from numerous other volunteers who have had primary trophozoite-induced malaria following injection of blood collected at a later stage of *P. vivax* malaria.

B. MALIGNANT TERTIAN MALARIA (NEW GUINEA STRAINS OF *P. falciparum*).

(1) SUBINOCULATIONS PERFORMED DURING THE FIRST 2 HOURS AFTER EXPOSURE OF THE DONOR TO INFECTIVE MOSQUITOES.

Donors were exposed to seven to twenty *A. punctulatus punctulatus* infected with viable sporozoites of New Guinea strains of *P. falciparum* at single sessions

lasting 7 to 15 minutes. The batches of mosquitoes were from 35 to 98 per cent infective, the gland infections were light-medium to heavy, and the sporozoite age was from 3 to 19 days.

The results obtained in these subinoculation experiments are epitomized in Table VI.

TABLE VI

*P. falciparum* MALARIA. SUBINOCULATIONS PERFORMED DURING THE FIRST 2 HOURS AFTER EXPOSURE OF DONORS TO INFECTIVE MOSQUITOES. SINGLE EXPOSURE ON DAY 0

Donor				Subinoculation			Recipient
Name	Number of infective bites	Duration of biting in minutes	First parasites in thick blood films (days after infection)	Minutes after biting ceased	Volume in c c	Result Positive (+) Negative (O)	First parasites in thick blood films (days after receiving blood)
Stw	12	5-8	9	during biting	500	O	0
Bri	18	8	*	during biting	500	+	11
Aus	12	5-8	11	7	500	+	13
Stu	15	15	10	10	500	O	0
Tre	7	7	11	15	500	O	0
McD	14	13	*	18	150	+	14
Stv	7	10	11	20	500	O	0
Ash.	24	8	9	60	500	+	12
Jac	18	8	8	60	500	O	0
O'D	20	6	(15)*	120	500	O	0

\* Given paludrine before the development of demonstrable parasites

#### (a) Primary attacks

It will be seen from data presented in Table VI that the presence of viable parasites in the peripheral circulation was demonstrated during the actual biting and 7, 18 and 60 minutes after cessation of biting. As with vivax malaria, it is assumed that these parasites were sporozoites.

The primary attacks of malaria in the positive recipients did not differ significantly from those of the donors beyond showing a longer incubation period. *P. falciparum* were demonstrated in the blood of donors between the 8th and 11th days after exposure, and between the 11th and 14th days after the recipients received their inocula.

No opportunity of studying secondary attacks of *falciparum* malaria was

forthcoming, since both the donors and recipients were radically cured by treatment during the primary attacks.

(b) *Atypical clinical syndrome associated with blood transfusion of sporozoites (P. falciparum).*

One recipient (Woo.) of 500 c.c. blood transfused 2 hours after exposure of the donor (O'D.) to infective bites (*P. falciparum*) developed a similar syndrome to that already described in the section on vivax malaria (volunteer Bun.)

Volunteer (Woo.) developed splenomegaly left shift of the neutrophil leucocytes, and mild fever. Repeated examinations of 1 c.mm. of blood in thick films never revealed *P. falciparum* parasites.

Subinoculation of 200 c.c. whole blood on the 53rd day after the initial exposure was negative and the condition terminated in natural cure. His case history is appended.

VOLUNTEER—WOO (CHART 8).

*Infection.*—This volunteer received 500 c.c. of whole blood by direct transfusion from donor O'D. 2 hours after biting. Donor O'D. was bitten by twenty *A. punctulatus punctulatus* infected with viable *P. falciparum* sporozoites. The sporozoites age was 10 days, the salivary glands heavily infected and the batch used was 95 per cent. infected. The donor O'D. developed demonstrable blood parasites on the 15th day.

*Course.*—During the first 3 weeks after receiving the inoculum the volunteer had occasional rises of temperature to 99° F but there was no tenderness of spleen or liver. H. complained of no symptoms. Parasites were not demonstrable (Chart 8.)

On the 24th day there was rise of temperature to 100.4° F but the volunteer complained of no constitutional symptoms. H. did, however experience dragging sensation in the left hypochondrium. The spleen was palpable with its lower border one finger breadth below the costal margin at the end of deep inspiration. The spleen remained palpable until the 29th day. There were no further symptoms during this period and the temperature did not again rise above 99° F. There was significant left shift (polymorphonuclear leucocytes on the 24th day) finding that has commonly been observed at certain stages in malaria infection. No parasites were demonstrated in thick blood films (1 c.mm.) which were examined daily from the 11th to 30th days.

During the next 10 days there were frequent rises of temperature to 99° F to 99.8° F but no symptoms were recorded. No parasites were demonstrated in thick films (1 c.mm.) The left shift of the neutrophils was not present on the 28th and 35th days when these examinations were made.

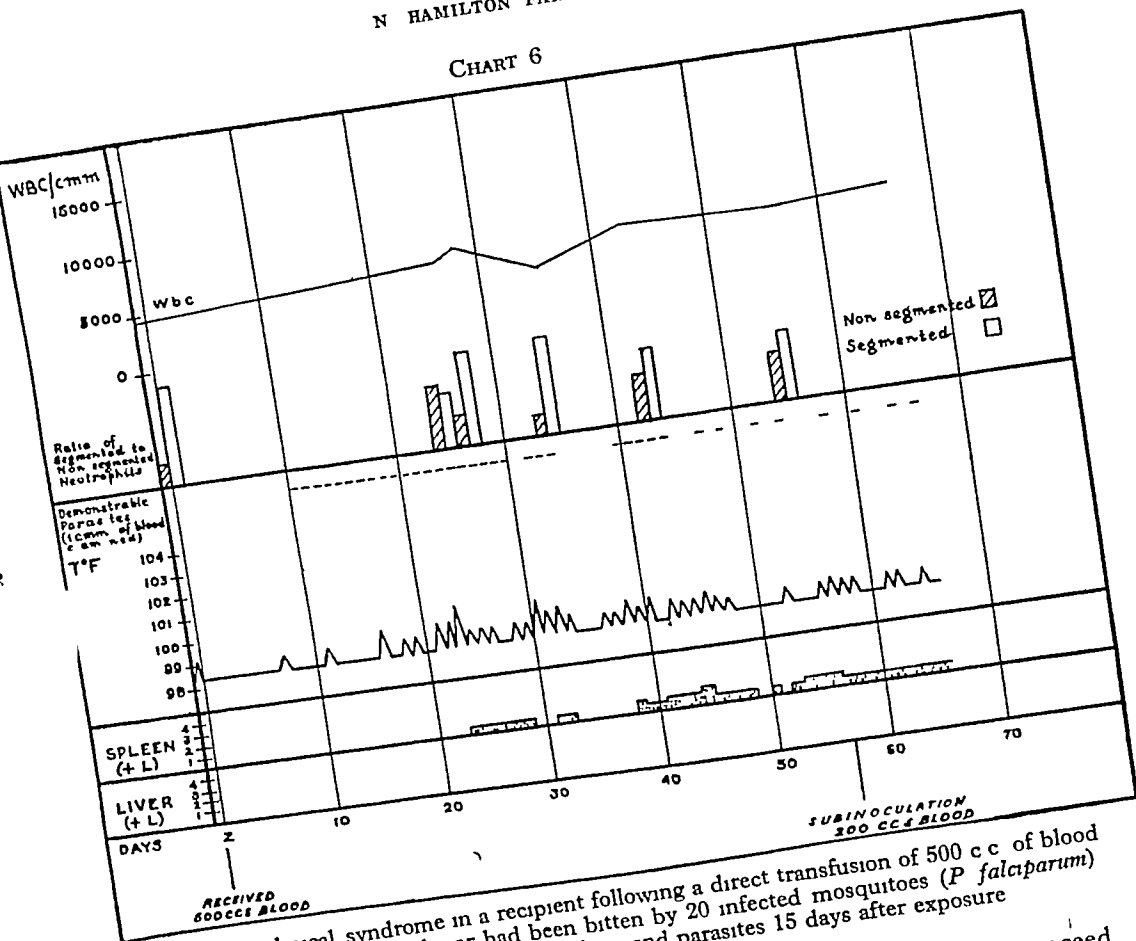
The spleen again became palpable on the 39th day and remained so up till the end of the 8th week. It varied in size from being just palpable at the costal margin to slightly more than one finger breadth below the costal margin. There were occasional rises of temperature to 99° F but no symptoms. The left shift in the polymorphonuclear leucocytes reappeared, being observed on the 43rd and 55th days. No parasites were demonstrated in thick blood films (1 c.mm.) examined daily from the 40th to 44th day. Subsequently blood films were collected daily but only examined twice weekly.

On the 53rd day 200 c.c. whole blood was transfused into another volunteer who developed no evidence of malaria infection. The spleen remained palpable until the 66th day and no parasites were ever demonstrated in thick blood films.

As in the case of Bun. (vivax infection) the hypothesis suggested in volunteer O.D. (*falciparum* infection) is that the sporozoites had become attenuated and

N HAMILTON FAIRLEY

CHART 6



Atypical clinical syndrome in a recipient following a direct transfusion of 500 c.c. of blood made 2 hours after the donor had been bitten by 20 infected mosquitoes (*P. falciparum*). The donor developed overt malaria and parasites 15 days after exposure.

modified by transfusion so that the exoerythrocytic schizogony did not proceed beyond the macroschizont and macromerozoite stage, or, if it did, the resulting micromerozoites were so few in number and so devitalized that they failed both to attain a density demonstrable microscopically and to establish the erythrocytic asexual cycle. This would suffice to explain the development of splenomegaly in the absence of demonstrable blood parasites and overt attacks. The spontaneous disappearance of e.e. forms later would result in natural cure.

(2) SUBINOCULATIONS PERFORMED DURING THE FIRST 9 DAYS AFTER EXPOSURE OF THE DONOR TO INFECTIVE MOSQUITOES (*P. falciparum*)

Two groups of donors were investigated, the first, group C, received no drug treatment until after overt malaria had developed, the second, group D, were taking atabrin or other schizonticidal anti-malaria drugs in the usual

suppressive dosage adopted in the previous experiments. This was 100 mg. daily for atabrin, resochin or santonin, and 20 grains daily for quinine.

(a) Group C—Controls

The donors in Group C were exposed to seven to 100 *A. punctulatus punctulatus* infected with viable sporozoites of New Guinea strains of *P. falciparum* at single sessions lasting 7 to 11 minutes. The batches of mosquitoes were from 35 to 100 per cent. infective—the gland infections varied from light medium to very heavy and the sporozoite age from 1 to 19 days.

The results obtained in these subinoculation experiments are epitomized in Table VII.

TABLE VII.

GROUP C—CONTROLS. SUBINOCULATIONS PERFORMED DURING THE FIRST 9 DAYS AFTER EXPOSURE OF THE DONOR TO INFECTIVE MOSQUITOES (*P. falciparum*). SINGLE EXPOSURE ON DAY 0.

Donor		Subinoculation			Recipient	
Name.	Number of infective bites	First parasites in thick blood films (days after infection).	Time after biting ceased (days/films)	Volume in c.c.	Result. Positive (+). Negative (0).	First parasites in thick blood films (days after receiving blood).
Stw	12	8	1	500	0	0
Aus.	12	11	2	500	0	0
Tre.	7	11	3	500	0	0
Fa.	8	10	4	200	0	0
Bak.	100	8	4/16	500	0	0
Ken.	20	7	8	500	0	0
Cro.	20	8	8/16	200	0	0
Rim.	19	7	8/16	200	0	0
Bak.	100	8	8/16	500	0	0
Fa.	8	10	8/20	200	0	0
OTD.	20	(18)	8	500	0	0
Ity	19	8	8	500	0	0
Cro.	20	8	6/16	200	+	4
Rus.	19	7	6/16	200	+	4
Rab.	20	8	6/16	20	+	7
Rim.	19	7	7/16	200	+	4
Stw	7	11	8/14	200	+	8

No treatment was given to any of these volunteers until overt malaria had developed, except Rob. who received paludrine on the 7th day after exposure.

It will be seen from a perusal of Table VII that subinoculations made from 1 to 6 days after exposure of the donors to seven to 100 infective bites (*P. falciparum*) were negative in twelve out of twelve instances. All twelve original donors developed falciparum malaria, parasites being demonstrated in thick smears from 7 to 15 days after exposure. On the other hand, it will be noted that in five out of five volunteers receiving seven to twenty infective bites, subinoculations made 160 to 206 hours after exposure were all positive, the recipients invariably developing falciparum malaria.

(b) *Group D—Receiving suppressive schizonticidal drugs*

These volunteers were bitten by two to twenty *A. punctulatus punctulatus* with viable sporozoites of *P. falciparum* (New Guinea strains) in their salivary glands. The batches were from 60 to 100 per cent infective, the gland infections light to heavy, and the sporozoite age varied from 2 to 13 days.

The detailed results in Group D are given in the accompanying Table VIII.

It will be seen that subinoculations made 5 days and 14 hours after exposure of the donor to ten infective bites (*P. falciparum*) were negative in two out of two volunteers taking suppressive atabrin. In contrast to this it will be observed that in twenty-nine out of twenty-nine volunteers receiving a variable number of infective bites (one to twenty) while taking suppressive drugs, subinoculations made from 158 to 208 hours after exposure were invariably positive, the recipients all developed overt falciparum malaria.

(c) *Combined results*

The results of subinoculations obtained in these two groups C and D detailed in Table VII and Table VIII have been condensed in Table IX (p. 647).

It will be seen from Table IX that the blood of fourteen out of fourteen volunteers collected 1 to 6 days after exposure to malaria infection failed to transmit malaria to the recipients, while the blood of thirty-five out of thirty-five volunteers collected from 6½ to 8½ days after infection invariably produced overt malaria in the recipients.

The earliest *positive* subinoculation was obtained on the 7th day—i.e., 160 hours after exposure. The latest *negative* subinoculations were obtained on the 6th day—144 hours after exposure. Thus it would appear that the first erythrocytic parasites of *P. falciparum* (micromerozoites) were first released into the circulation between 144 and 160 hours after exposure to infective mosquitoes.

In these mosquito-transmitted falciparum infections it would appear that sporozoites gaining access to the circulation are effectively removed by the reticulo-endothelium, here analogy with avian malaria suggests they undergo an intracellular schizogonous cycle with the production of cryptozoites and

TABLE VIII.

GROUP D. VOLUNTEERS INFECTED WITH *P. falciparum* WHILE HAVING QUININE, SULPHADIAZINE, SONTOSCHIN, OR RESOCHIN IN SUPPRESSIVE DOSAGES.  
SUBINOCULATIONS PERFORMED BETWEEN THE 5TH AND 9TH DAYS AFTER EXPOSURE.  
(SINGLE EXPOSURE ON DAY 0)

Donor					Subinoculation			Recipient
Name.	Drug	Number of infective bites.	First parasites in thick blood films (during suppression)	Overt malaria after ceasing suppression.	Subinoculation after exposure (days/hours).	Volumes in c.	Result.	First parasites in thick blood films (days after receiving blood).
Kae	Atebrin	10	0	O	5/14	200	O	0
Cal	Atebrin	10	0	O	8/14	200	O	0
Kae	Atebrin	10	0	O	6/14	200	+	6
Cal	Atebrin	10	0	O	6/14	200	+	8
Rya	Atebrin	20	0	O	6/14	200	+	8
Sta.	Atebrin	2	0	O	6/14	200	+	8
Gob	Atebrin	2	0	O	6/14	200	+	6
Mil.	Atebrin	2	18	+†	6/14	200	+	7
Law	Quinine	11	0	O	6/14	200	+	7
Nae.	Quinine	10	0	O	6/14	200	+	8
Chu.	Sulphadiazine	10	0	O	6/14	200	+	4
Hod.	Atebrin	10	0	O	7/14	200	+	6
Kae	Atebrin	10	0	O	7/14	20	+	6
Cal.	Atebrin	10	0	O	7/14	20	+	6
Ree	Atebrin	7	20	+†	7/16	20	+	8
Law	Atebrin	20	0	O	7/16	200	+	6
Sta.	Atebrin	20	0	O	7/16	200	+	8
Fre.	Sontoschin	20	0	O	7/16	200	+	8
Dr.	Sontoschin	20	0	O	7/17	200	+	8
Fug.	Sontoschin	20	0	O	7/17	200	+	8
Nae.	Sontoschin	20	0	O	7/17	200	+	8
Rat.	Atebrin	1 2	0	O	7/18	200	+	10
Vid.	Sontoschin	1 2	(14)*	O	7/19	200	+	11
Wak.	Resochin	20	0	O	7/19	200	+	8
Nae.	Resochin	10	0	O	7/20	200	+	8
Tho.	Atebrin	10	0	O	8/14	500	+	9
Hat.	Atebrin	26	18	+†	8/14	200	+	8
Fre.	Sontoschin	20	0	O	8/16	200	+	12
Dr.	Sontoschin	20	0	O	8/16	200	+	12
Fug.	Sontoschin	20	0	O	8/16	200	+	10
Nae.	Sontoschin	20	0	O	8/16	200	+	10

+ = positive. O = negative. † Single ring trophozoite of *P. falciparum*

† A relatively atebri-resistant strain of *P. falciparum* from Acapulco-Wewak area of New Guinea was employed to infect these volunteers. All others were infected with the usual atebri-susceptible strains.

TABLE IX

RESULTS OF SUBINOCULATIONS PERFORMED BETWEEN THE 1ST AND 9TH DAYS AFTER EXPOSURE OF FORTY-NINE VOLUNTEERS TO MOSQUITOES INFECTIVE WITH *P. falciparum* (GROUPS C AND D)

Days since exposure of donor	Group C			Group D		
	Number of sub-inoculations	Results		Number of sub-inoculations	Results	
		Positive	Negative		Positive	Negative
1	1	0	1	—	—	—
2	1	0	1	—	—	—
3	1	0	1	—	—	—
4	1	0	1	—	—	—
4½	1	0	1	—	—	—
5	1	0	1	—	—	—
5½	4	0	4	2	0	2
6	2	0	2	0	0	0
6½	4	4	0	9	9	0
7½	1	1	0	14	14	0
8½	1	1	0	6	6	0
Total	18	6	12	31	29	2

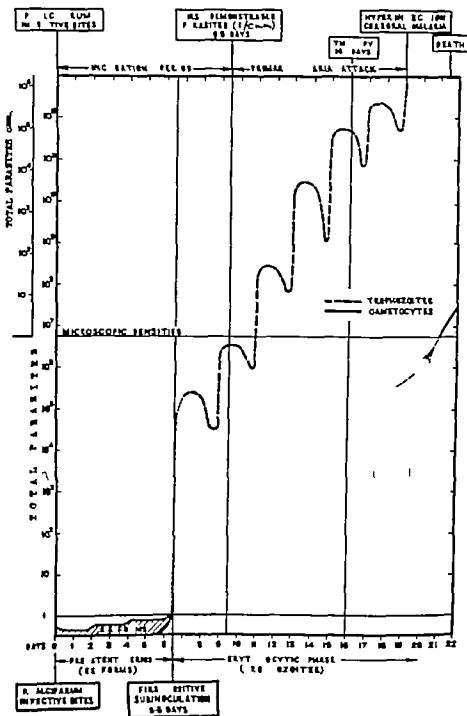
metacryptozoites. If the intracellular schizogonous cycle lasts 48 hours there would be three complete cycles before the first erythrocytic parasites (micro-merozoites) escape into the blood stream (Chart 7, p 648)

The following observations were made on one volunteer, Rim (Group C) exposed to nineteen infective mosquitoes

- (1) 136 hours after exposure—negative subinoculation
- (2) 160 hours after exposure—positive subinoculation
- (3) 164 hours after exposure—ring forms of *P. falciparum* demonstrated in thick blood films to the order of 1 per c mm, i.e., a negative sub-inoculation (using 200 c c of whole blood) was obtained 28 hours prior to the demonstration of *P. falciparum* to the order of 1 per c mm in the peripheral circulation

The observations on this volunteer strongly suggest that there must be a large number of erythrocytic parasites (micromerozoites) released from the schizogonous cycle of pre-erythrocytic forms at the end of the prepatent period. In some instances, this outflow would be of the order of 1,000,000 to 5,000,000, though it is considered that more usually there would be 200,000 to 500,000 erythrocytic parasites liberated at the end of the prepatent period.





Note that pre-erythrocytic or hypothetical a.s. forms are considered not to persist after 6 days being completely transformed into erythrocytic parasites (microgametocytes). The erythrocytic parasites steadily increases from subinoculation characteristically to hyper-infection; the fall in the parasite curve during each cycle is due to the larger segmented erythrocytic parasites being withdrawn from the circulating blood.



number of vivax parasites resulting. The complete absence of demonstrable vivax parasites in thick blood films in two out of three volunteers exposed on day 0 suggests the latter possibility. Similarly in natural infections with the two species of *Plasmodium* it is rare to find vivax and falciparum parasites together in thick blood films.

In the earlier stages the clinical attacks in mixed infections did not materially differ from infections with one or other species of parasite, though when trophozoites of both species were present together the attacks were naturally severe.

Secondary attacks occurred in three out of five volunteers. They were associated exclusively with *P. vivax* parasites. The two infected volunteers who failed to develop secondary attacks were observed over a period of 349 and 640 days respectively.

While taking suppressive atebirin, a sixth volunteer was exposed to both falciparum and vivax parasites on day 0. Suppression of his double infection was complete and no parasites were demonstrable in blood films while taking atebirin. Subinoculation on the 8th day however produced falciparum malaria in the recipient while a second subinoculation on the 10th day in another volunteer produced an overt attack with parasites of *P. vivax* and *P. falciparum* demonstrable in thick blood smears.

The results of subinoculations in this volunteer are of considerable interest. The case history is given below (Chart 8).

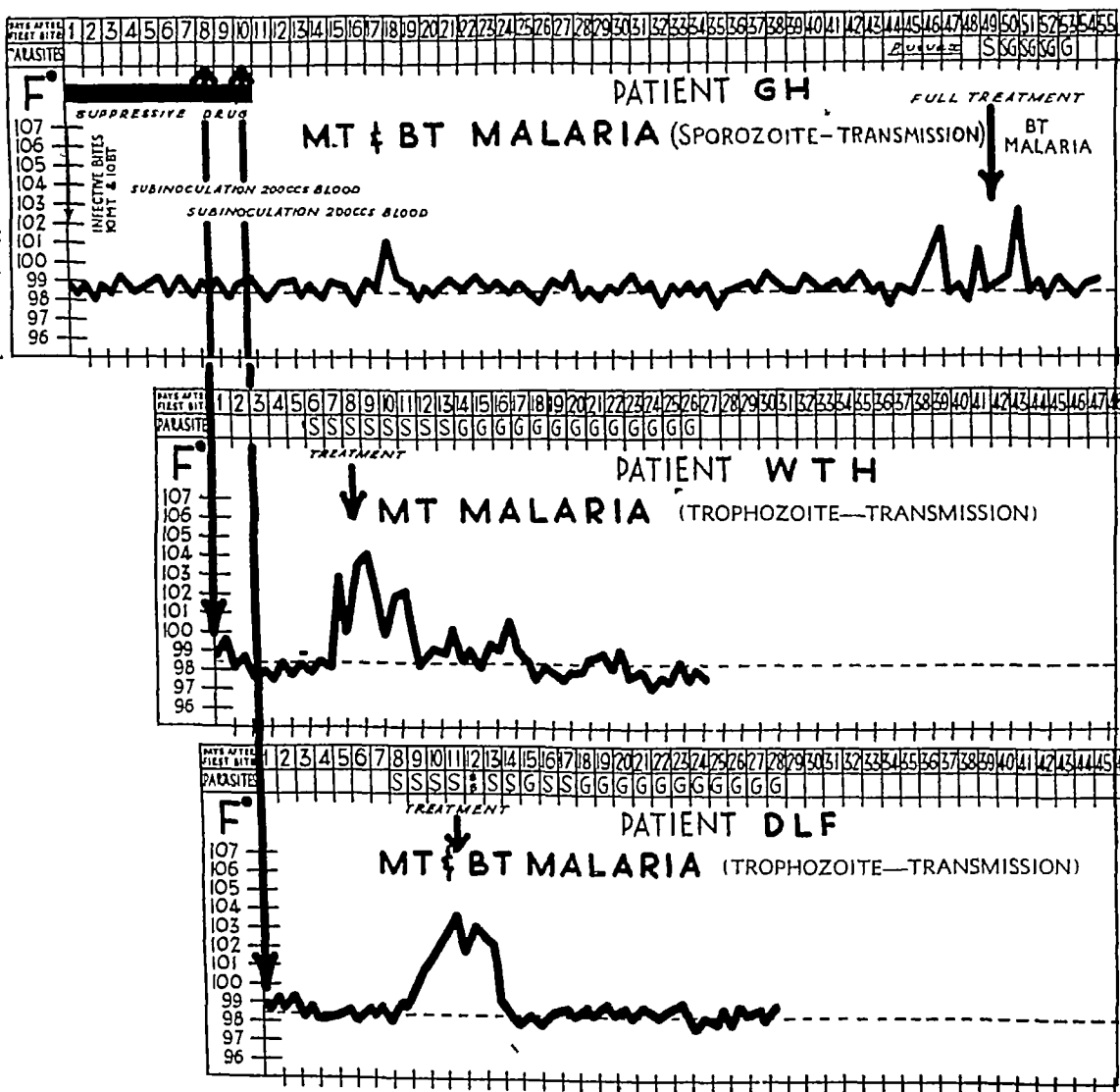
A volunteer G.H. was exposed to malaria infection while taking atebirin. He had received 0.4 grammes daily for 4 days prior to exposure to infection in order to build up an adequate concentration of atebirin in the blood. The drug was continued in dosage of 100 mg. daily for the next 10 days. On zero day he received twenty infective bites, ten from *A. punctulatus punctulatus* harbouring sporozoites of *P. falciparum* in the salivary gland and ten from another batch of anopheline mosquitoes infected with *P. vivax* sporozoites.

There was a transient rise of temperature to 100° F. on the 17th day unassociated with demonstrable parasites in blood smears and thereafter no significant rise of temperature was recorded until the 46th day when fever of tertian type developed. Parasites of *P. vivax* were demonstrated on the 49th day when full course of quinine-atebirin-plasmoquine treatment was instituted. (Chart 8.) The concentration of plasma atebirin on the 8th day was 35 microgrammes, on the 11th day 30 microgrammes, on the 14th day 28 microgrammes and on the 16th day 10 microgrammes per litre. Evidently this concentration was adequate to produce a radical cure of the falciparum infection in the donor even though atebirin was only administered for the first 10 days after exposure to infection. It failed, however, to eradicate the vivax infection.

On the 8th day following exposure to infection direct transfusion of 200 c.c. of blood from volunteer G.H. was given to volunteer W.T.H. Overt malaria developed 8 days later associated with parasites of *P. falciparum* in thick blood smears. Treatment commenced 2 days later. A gametocyte wave followed commencing on the 14th day; by this time the asexual erythrocytic parasites had disappeared. No clinical or parasitological evidence of vivax malaria ever developed in this volunteer (Chart 8).

On the 10th day following exposure to infection, a similar direct transfusion of 200 c.c. of blood from volunteer G.H. was given to volunteer D.L.F. Overt malaria with demonstrable asexual parasites developed on the 8th day and microscopical examination subsequently revealed the presence of both *P. falciparum* and *P. vivax*. Treatment commenced on the 11th day. Gametocytes first appeared on the 15th day and were constantly present from the 18th to the 23rd day when examination ceased. (Chart 8.)

## CHART 8

SUBINOCULATIONS FOLLOWING MIXED INFECTION (*P. falciparum* AND *P. vivax*)

*Comment*—It is evident from this and other results that in simultaneous infection with sporozoite-transmitted falciparum and vivax malaria, the blood of the exposed donor contains only parasites of *P. falciparum* on the 8th day whereas by the 10th day parasites of *P. vivax* are also present—though only in submicroscopic density. The result constitutes a striking verification of the findings already recorded in volunteers experimentally infected with either *P. falciparum* or *P. vivax* species of parasite.

#### (D) PREMONITORY CLINICAL FEATURES ASSOCIATED WITH SUB MICROSCOPIC DENSITIES OF PARASITES.

On an average parasites are demonstrable microscopically 9½ days after infection with *P. falciparum* and 12 days with *P. vivax* even when as much as 1 c.mm. of blood is examined in thick smears stained by Field's method. There is thus a period of about 3 days in which parasites are present in a submicroscopical density which can only be revealed by subinoculation. Throughout this period minor clinical features were frequently recorded in these volunteers associated with certain haematological changes characterized by a decrease in lymphocytes, left polymorphonuclear shift and relative leucopenia. Subjective symptoms include transient headache, backache and generalized aches and pains. Abdominal examination sometimes reveals tenderness over the liver and in the vicinity of the gall bladder. A slight temperature of 99° F. on one or more days was not uncommon. These minor symptoms were transient in experimentally infected volunteers taking suppressive drugs like atabrin in adequate dosage. In the controls, however, who were receiving no drug they increased in intensity with the onset of the overt attack when major symptoms developed and parasites became demonstrable in blood smears. These premonitory clinical symptoms, though mild and transient, were so frequent both in control volunteers and those on suppressive schizonticidal drugs of the atabrin type, and the haematological features were so constant, that it is difficult not to associate their origin with the presence of submicroscopic densities of parasites which can constantly be revealed by subinoculation.

### SECTION II.

#### SUBINOCULATION AS AN INDEX TO THE ACTION OF ANTI MALARIAL DRUGS

There are several possible stages in the life history of malaria parasites in man and the mosquito which may be variably affected by anti-malaria drugs. These stages are —

- (1) Sporozoites.
- (2) The pre-erythrocytic or early e.c. forms (cryptozoites and meta-cryptozoites).
- (3) Asexual erythrocytic parasites—merozoites, trophozoites, schizonts.



with mathematical regularity though at this stage they were generally present only in submicroscopic density.

*Late e.e. Forms.*—There is evidence that in recurrent vivax malaria plasmoquine may produce radical cure by its action on late e.e. forms. SEXTON and BIRD (1928) showed that radical cure is best achieved in vivax malaria by an intensive course of plasmoquine in combination with quinine given during a febrile attack. FAIRLEY and his colleagues (1946) obtained comparable results with a combined course of plasmoquine and paludrine in relapsing vivax malaria but paludrine alone—even in a dosage of 1.0 gramme daily for 14 days—failed to produce radical cure in vivax infections. With a small maintenance dose of 100 mg. of paludrine twice weekly for 5 months the confirmed relapse rate in recurrent vivax infections has not exceeded 2.4 per 1,000 during the period of paludrine administration, but many more months will need to elapse after cessation of the drug before the proportion of radical cures can be even approximately assessed.

*Gametocytes and the Sexual Cycle*—Vivax gametocytes appear approximately 16 days after subinoculation of sporozoites (Chart 5) *i.e.* 4 to 5 days after asexual parasites are demonstrable in thick smears. Falciparum gametocytes, on the other hand, do not generally appear until about the 21st day after inoculation of sporozoites (Chart 7) *i.e.* some 10 days after asexual parasites are first found in thick smears. Without a reasonable trophozoite density the secondary gametocyte wave is generally unsatisfactory. Accordingly experiments designed to study true gametocidal action have to be so timed as to determine the direct effect of the drug as a primary gametocide and not its secondary gametocidal effects dependent on schizonticidal action. An investigation of the subsequent development of gametocytes in the vector may also be necessary as shown by MACKERRAS and ERCOLE at Cairns: they found that after feeding mosquitoes on a carrier whose blood contains paludrine the paludrine inhibits development of oöcytes and/or vermicules within the stomach of the engorged mosquito even though this drug exerts no deleterious action on gametocytes in the peripheral blood of the human carrier. As the gametocidal action of anti malaria drugs is being considered elsewhere no further account here is necessary.

The data which follow are mainly concerned with the value of subinoculation in determining the mode of action of suppressant drugs, with special reference to schizonticidal action and causal prophylactic effects, and will be reviewed from this standpoint.

#### A. SCHIZONTICIDES.

##### (1) MALIGNANT TERTIAN MALARIA.

##### (a) Sporozoite-induced falciparum malaria.

During the course of these investigations it was found that when atebria was given in dosage of 0.1 gramme daily for suppressive purposes only minor

clinical symptoms developed,\* parasites were rarely demonstrable in thick blood films and the great majority of volunteers exposed to heavy and repeated infections with *P falciparum* were radically cured provided administration of the drug was continued daily for a period of 3 to 4 weeks after the last exposure to infection. It was also found in these infected volunteers that though parasites were not demonstrable microscopically, subinoculations made between 6½ to 8½ days invariably produced overt malaria in the recipients (Table VIII). When subinoculations were delayed for another 5 days or longer in infected volunteers taking suppressive atebirin negative results were obtained. Thus in a consecutive series of twenty-six such volunteers whose blood was collected 11½ to 70 days after exposure to *P falciparum*, the twenty-six recipients failed to get malaria, and further investigation revealed that while atebirin does not prevent viable asexual parasites reaching the blood stream, it exerts its curative effects as a schizonticide from the time the first generation of erythrocytic parasites appear, provided it is present in sufficient concentration in the blood. Subinoculation experiments indicate that it takes 3 to 5 days for malignant tertian parasites to be completely eradicated from the circulating blood under such circumstances and in these infections once a negative subinoculation has been established, experimentally infected volunteers have invariably proved to be radically cured (Chart 9, p 656).

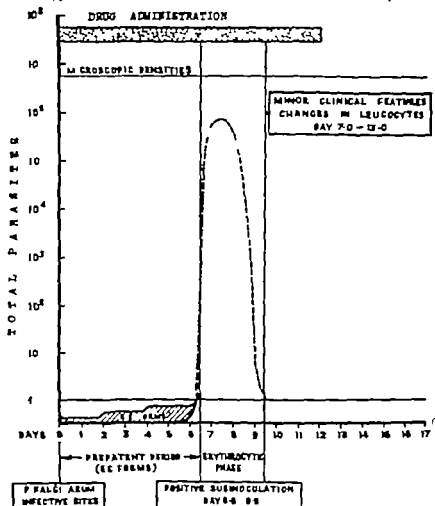
It was also demonstrated in the course of these experimental studies that a number of other drugs acted in an identical fashion when administered each day for suppressive purposes. These included certain sulphonamide drugs, namely, sulphadiazine, sulphamezathine and sulphamerazine given in a dosage of 10 grammes daily. It was found that six out of six volunteers taking sulphadiazine, four out of four taking sulphamezathine and eight out of eight taking sulphamerazine who had been heavily infected with *P falciparum* gave negative subinoculations from the 12th to the 70th day, and subsequent investigation proved all cured. Subinoculation on the 7th day from a volunteer taking sulphadiazine was positive, showing that asexual parasites were appearing in the blood stream normally and were later eradicated as a result of the schizonticidal action of this drug.

Sontochin (SN 6911) and resoquin (SN 7618), now known in the U.S.A. as chloroquine or aralen, are 4-amino quinoline compounds, which were synthesized and patented by the Germans in 1939 before the outbreak of war. At that time their pharmacology and therapeutic action in malaria had been very inadequately investigated. During the war, this group of drugs was intensively studied in the U.S.A. and early in 1944 both these drugs were sent by the National Research Council to be tested on experimentally infected.

\* A relatively atebirin-resistant strain, which was not suppressed by atebirin when given in a dosage of 0.1 gramme daily, was obtained from a carefully selected group of patients at Wewak. The results of this investigation were reported fully by FAIRLEY *et al* (1946) *Trans R Soc trop Med Hyg*, 40, 229.



CHART 9  
ACTION OF SUPPRESSIVE DRUGS IN SPOROBLITE-INDUCED MALIGNANT TERTIAN  
MALARIA IN MAN (*P. falciparum*).  
(QUININE, ATHERIN, BONTODIN, BEROCHIN, SULPHONAMIDES.)



Note that the hypothetical e.c. forms are regarded as not persisting beyond the pre-erythrocytic stage. Once the blood has been completely cleared of asexual parasites radical cure results.

volunteers at Cairns for their value as suppressants and possible causal prophylactic action.

While these drugs were being taken regularly the transfusion of 200 c.c. of blood from seven volunteers made on the 8th day and of four made on the 9th day

after exposure produced overt falciparum malaria in the recipients (Table VIII). The blood of seven out of seven similar volunteers taking suppressive santonin subinoculated 12 days after infection failed to produce malaria in the seven recipients. These and other experimental results in volunteers at Cairns showed that, like atabrin, both santonin and resochin suppressed and finally cured falciparum infections by schizonticidal action when given in a dosage of 0.1 gramme daily. Neither santonin nor resochin exerted a causal prophylactic action on sporozoites or on pre-erythrocytic or early e.c. forms, since subinoculations on the 8th or 9th day were invariably positive. In a few cases later observations showed that with resochin similar results were obtained in experimentally infected volunteers when the dosage was reduced to 50 mg daily. The effects on the asexual parasites obtained in these experiments with schizonticidal suppressive drugs are diagrammatically presented in Chart 9.

(b) *Trophozoite-induced falciparum malaria*

While atabrin or certain sulphonamide drugs were being administered in suppressive dosage, volunteers were given intramuscular or intravenous inocula of whole blood containing trophozoites of *P. falciparum*. The observations recorded in the previous paragraphs were amply confirmed. Suppression of primary fever was satisfactory and after cessation of drug administration overt attacks failed to develop. Twenty-five out of twenty-five subinoculations performed between 37 and 60 days after cessation of the suppressive drug regimens were negative. This indicated that the blood had been completely cleared of malaria parasites. If latent infection had persisted despite absence of demonstrable parasites in blood smears and negative subinoculations some degree of premunity might be anticipated. The twenty-five original volunteers were therefore injected with blood containing a known number of parasites (*P. falciparum*), in every instance overt falciparum malaria developed showing that in none of these volunteers was there effective premunity. Negative subinoculation and absence of premunity as judged by susceptibility to re-infection constitute the strongest evidence of radical cure.

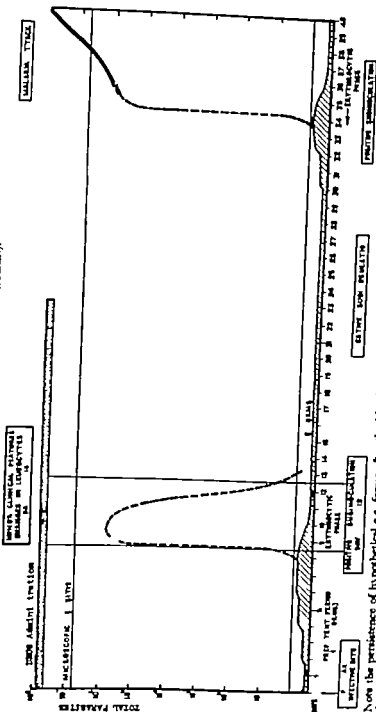
(2) BENIGN TERTIAN MALARIA (NEW GUINEA STRAINS)

(a) *Sporozoite-induced vivax malaria*

In *P. vivax* experimentally induced infections, atabrin in a dosage of 0.4 to 0.7 gramme weekly was found to suppress benign tertian malaria completely while the drug was being taken, quite irrespective of how often and how intensely the volunteers were bitten. In contradistinction to the findings in malignant tertian malaria infections, some 40 days after ceasing the drug overt benign tertian malaria developed in 97 per cent of such volunteers (Chart 10).

## CHART 10.

ACTION OF SUPPRESSIVE DRUGS IN STROKES IN INDIVIDUALS WITH MALARIA IN MALAYA (P. SINGH)  
(QUININE, ATHERIN, MONTOCHIN, MONOCHIN).



Note the persistence of hypothetical s.c. forms after the blood has been completely cleared of actual parasites by these schizonticidal drugs as judged by subinoculation; subsequently erythrocytic vivax parasites reappear first in subinoculation by positive subinoculation on overt attack follows at viable times after cessation of the drugs.

While suppressive atebryn was being taken and for some time later the careful and prolonged examination of thick blood smears had failed to reveal parasites, though subinoculation on the 9th to 11th days invariably resulted in an overt attack of vivax malaria developing in the recipient. Thus in a series of seven volunteers exposed to two to fifty infective bites (*P. vivax*) on zero day while taking 0.1 gramme of atebryn daily, subinoculations made 8 days 14 hours to 9 days 14 hours later were invariably positive (Table IV). Further experiments also showed it took 3 to 5 days to clear the blood of parasites with certainty as judged by subinoculation tests (Chart 10). In vivax infection, however, once a negative subinoculation had been established in a patient on suppressive atebryn or by curative therapy, it did not follow that he was cured. On the contrary, sooner or later positive subinoculations were obtained and in the vast majority of instances parasites were ultimately demonstrable microscopically and overt attacks ensued (Chart 10). The salient features revealed by multiple subinoculations during the course of suppressed vivax malaria are well illustrated in the following case history (Chart 11).

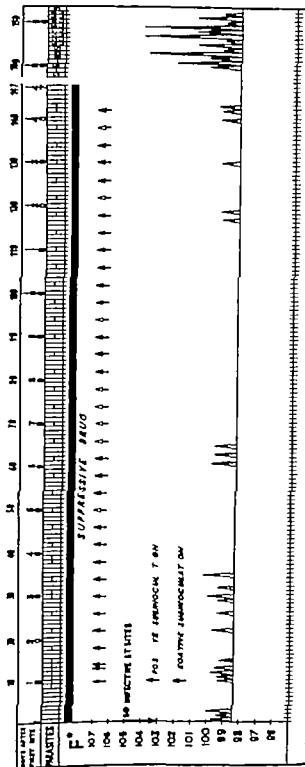
W.H.J.T. was given 0.4 gramme atebryn daily for 4 days and then received bites from fifty *A. punctulatus punctulatus* containing viable sporozoites in the salivary glands. He took 0.1 gramme atebryn for 147 consecutive days after exposure. Throughout this period there was no sustained pyrexia or splenomegaly and parasites were never found in thick blood smears only minor clinical features being noted. On the 178th day after exposure to infection, i.e., 31 days after ceasing to take atebryn, parasites were demonstrated microscopically and an attack of overt malaria developed a few days later. During the first 142 days following infection subinoculations were done at 4- to 5-day intervals, a total of thirty-five subinoculations being performed. The first two subinoculations on the 10th and 13th days were positive, from the 14th to the 38th day seven subinoculations were done with negative results. On the 42nd day a positive subinoculation was recorded, the recipient of 20 c.c. of blood developing typical vivax malaria. No parasites were demonstrable in 1 c.mm. of blood examined microscopically on that day. The inoculum consisted of 20 c.c. of blood except on the 13th and 42nd day when two subinoculations were done, one of 20 c.c. and the other of 200 c.c. On both these days, both recipients were positive. During the next 100 days twenty-five subinoculations were not performed after drug suppression ceased (Chart 11). Further inoculations were not performed after drug suppression ceased.

**Comment**—This experimental clinical study is of interest as it demonstrated that —

- (1) Asexual parasites were present in the blood from the 10th to the 13th day
- (2) Clearance of the parasites from the blood by suppressive atebryn resulted and lasted for a period of at least 24 days
- (3) Asexual parasites reappeared at a submicroscopic level on the 42nd day—a common time of relapse for New Guinea vivax strains. There was no associated fever and parasites could not be found after prolonged search of thick films collected that day

CHART 11

SUBINOCULATIONS IN AN INFECTED VOLUNTEER TAKING 100 MG. OF ATABRIN DAILY (P. vivax)



Subinoculations revealed that asexual parasites were present on the 10th and 13th day disappeared for a period of 24 days, reappeared temporarily in submicroscopic density on the 43rd day and subsequently failed to reappear throughout the period of drug administration. An overt attack of B.T. malaria developed 31 days after atabrin medication ceased.



TABLE X.

CURE RATE WITH TEBRIN IN SUPPRESSED AND FULLY TREATED SPOROZOITE AND TROPHOZOITE EXPERIMENTALLY INDUCED VIVAX MALARIA. (NEW GUINEA STRAINS)

Malaria transmission.	Suppressive stebrin.			Cure therapy		
	Total number.	Number cured.	Percentage cured.	Total number.	Number cured.	Percentage cured.
Sporozoite (mosquito-transmitted)	100	3	3	200	Less than 30	Less than 15
Trophozoite (blood inoculated)	9	9	(100)	90	84	93

with high dosages of quinine or stebrin, or the standard quinine stebrin and plasmoquine course relapses have occurred in at least 82 per cent. of the patients. The cure rate in vivax malaria did not exceed 18 per cent. and probably in the final analysis it will prove less than this when individual volunteers have been followed over a 2 year period.

### (3) DISCUSSION

Analyses of the data on subinoculation presented in this paper confirm that in malignant tertian malaria once the blood is cleared of parasites recrudescences do not occur and the infection is cured. From this viewpoint there is little, if any difference in the response to suppressive or curative therapy of sporozoite-induced and trophozoite-induced *P. falciparum* infections. The most likely explanation of these findings is that in *P. falciparum* infections, the activities of the pre-erythrocytic or early e.c. forms are confined to the production of asexual parasites (micromerozoites) responsible for the primary attack, and do not persist as late e.c. forms in the reticulo-endothelial cells (Chart 9).

In sporozoite or trophozoite-induced *P. vivax* infections on the other hand, the ease with which the blood is cleared of asexual parasites by treatment as indicated by negative subinoculations, and the high frequency of relapse in the sporozoite-induced infections supports the view that this is due to persistent e.c. forms whereby asexual forms of *P. vivax* (New Guinea strains) are produced generally at 4- to 8-week intervals at first and possibly later at longer and more irregular periods when the biological equilibrium between host and parasite has been disturbed.

Relapses rarely follow adequately treated trophozoite-induced vivax malaria: this suggests that e.c. forms in *P. vivax* are derived exclusively from the pre-erythrocytic parasites and cannot be formed later from asexual invading tissue cells (Chart 10 p. 658).

During the latent period when subinoculations are negative, it is evident that in drug-suppressed vivax infections viable first generation merozoites (micromerozoites) are failing to reach the blood stream for quite long intervals. The basis of this is not definitely known.

It may be that the e e forms are temporarily in a resting stage and schizogony is not proceeding at all, or there may be slowing down or partial inhibition of e e schizogony. It would also appear possible from analogy with *P. praecox* infection in birds (REICHNOW and MUDROW, 1943, 1944) that in latent vivax malaria the e e cycle does not proceed beyond the macroschizont and macromerozoite stages for considerable periods, and that microschizonts and micromerozoites may only be produced a few days before parasites are demonstrable in the blood by subinoculation or in blood smears.

In untreated vivax malaria which is allowed to run its natural febrile course with the development of premunity, vivax parasites may subsequently appear from time to time in readily demonstrable numbers in the blood without the development of overt attacks or major symptoms. Under these circumstances the density of erythrocytic parasites is effectively controlled by an acquired immunization mechanism, the parasitized cells being rapidly phagocytosed as a result of the combined action of specific opsonins and hypertrophied reticulo-endothelial tissue. Biological equilibrium between host and parasite has been achieved.

### (B) CAUSAL PROPHYLACTICS

Strictly speaking, the term causal prophylactic should only be applied to a drug which destroys malaria sporozoites on inoculation and prevents further cyclic development in man. In this paper, the term is used in the sense employed by DAVEY (1946), namely, a drug which prevents malaria parasites ever appearing in the red blood cells. Such a drug may act on the sporozoite or on the pre-erythrocytic stages, *ie*, the early e e forms (cryptozoites and metacryptozoites) existing between the sporozoite and the blood parasites. The term partial causal prophylactic is applied to a drug which inhibits the production of asexual erythrocytic parasites during the period of its administration but fails to prevent an overt attack after drug administration ceases.

None of the drugs so far discussed are true causal prophylactics in either *P. vivax* or *P. falciparum* infections. It has been shown during the course of these experiments that the first generation of asexual erythrocytic parasites are liberated with mathematical precision into the blood on the 7th day in falciparum and the 9th day in vivax infections despite the fact that atabrin, sonotochin, resochin, quinine or sulphadiazine or allied compounds are given in appropriate dosage each day. Clinically, these schizonticides are exerting no effect either on the sporozoites or pre-erythrocytic or early e e forms, for the appearance of asexual parasites is not delayed. Effective as atabrin and some of these other drugs are as suppressants, they do not constitute the ideal pro-



phylactic drug for malaria since they do not prevent the occurrence of the asexual parasites in the blood.

Similarly if as postulated, relapses in *P. vivax* are due to the persistence of e.c. forms, cure will only be attained with certainty by a drug acting on these late e.c. forms. Schizontocidal action is adequate for suppression and clinical cure but not for radical cure.

In experimentally infected volunteers a number of anti malaria drugs have been investigated for

(1) Causal prophylactic action resulting from destruction of the sporozoites or early e.c. (pre-erythrocytic) forms

(2) Radical cure attributable to the destruction of late e.c. forms.

At least two groups of drugs have been definitely shown to possess a causal prophylactic action in falciparum infections in man namely the 8-amino quinolines of which plasmoquine is the best known example, and the biguanides of which paludrine is the outstanding example.

## 1 THE 8-AMINO QUINOLINES.

### Plasmoquine.

JAMES (1935) first demonstrated plasmoquine to be a true causal prophylactic in malignant tertian malaria using a Roumanian strain of *P. falciparum*. Five out of five volunteers, receiving 0.8 gramme of plasmoquine base daily for 8 days commencing the day before infection, failed to develop overt malaria within a period of 14 months.

Three patients were given 0.8 gramme of plasmoquine base on the evening before infection (*P. vivax*) and the same dose on the next day at the actual time of receiving five to ten infective bites. One patient developed parasites (*P. vivax*) in the blood after 16 days and a clinical relapse 18 days later. The other two developed their first attack of vivax malaria 275 days and 328 days after infection.

(1) *P. falciparum* infection.—During the present researches a group of eight volunteers were exposed to the bites of twelve mosquitoes *A. punctulatus punctulatus* whose salivary glands contained viable sporozoites of *P. falciparum*. Four volunteers received plasmoquine base in a dosage of 80 mg. on the day prior to exposure, on the day of exposure and for 5 days subsequently. Subinoculations performed 7½ and 8½ days after exposure were negative in three instances and positive in one. Subsequent investigation showed that the three volunteers, 200 c.c. of whose blood failed to produce malaria in the three recipients, were completely cured, whereas the volunteer giving the positive subinoculation developed a slightly delayed overt attack within the usual incubation period of malignant tertian malaria.

Another four volunteers who were similarly infected were given plasmoquine base in a dosage of 80 mg. daily from the 6th to the 10th days inclusive. Two subinoculations (200 c.c.) were made on the 8th day and two on the 9th day with positive results. All four donors developed overt malaria.

*Comment*—These results indicated with this New Guinea strain of *P. falciparum* that plasmoquine in this large dosage acted as a true causal prophylactic, destroying the sporozoites or early e.e. (pre-erythrocytic) forms or both in three out of four instances (Chart 12). Its schizonticidal value was not satisfactory as in the same dosage it failed to clear the blood of asexual parasites and prevent overt malaria developing, when its administration was delayed until the 6th day.

(2) *P. vivax* infection.—Similar experiments were undertaken with other groups of volunteers infected with *P. vivax* malaria.

In the first group twenty *A. punctulatus punctulatus* harbouring the sporozoites of *P. vivax* in the salivary glands engorged on eight volunteers. Four of these received plasmoquine base in a dosage of 80 mg. on the day preceding exposure, on the day of exposure and for the subsequent 5 days. Two were subinoculated on the 9th day, and two on the 10th day following infection. The four recipients failed to get malaria but, despite this fact, the four donors later developed delayed attacks of overt malaria (Table XI).

In another similar experiment the remaining four volunteers took 80 mg. plasmoquine base daily from the 7th to the 11th day. Two subinoculations were made on the 9th day and two on the 10th day. All four recipients later developed overt vivax malaria, as did the four original donors (Table XI).

TABLE XI

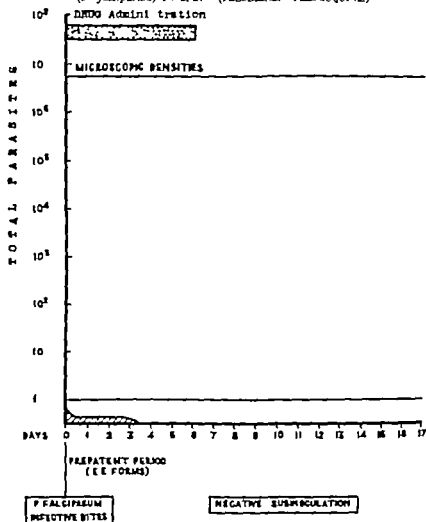
SUBINOCULATIONS FROM VOLUNTEERS RECEIVING PLASMOQUINE 80 MG. (AS BASE) DAILY AND EXPOSED TO EXPERIMENTAL MOSQUITO-TRANSMITTED *P. vivax*

Duration of plasmoquine administration (days*)	Number of volunteers	Subinoculations				Ultimate overt malaria
		Days performed	Number	Results †		
				+	O	
—1 to +5	4	+ 9 +10	2 2	1	2 2	4
0 to +8	1	+11 +15 +43	1 1 1		1 1 1	0
+6 to +10	4	9 +10	2 2	2 2		4

\* Day —1 = day before exposure to infection, day 0 = day of exposure to infection, day +1, etc. = 1 etc. day after exposure to infection.

† + = positive result O = negative result

CHART 12.

ACTION OF CAUSAL PROPHYLACTIC DRUGS IN SPOOROZOITE INDUCED MALARIA (*P. falciparum*) IN MAN (PALUDRINE, PLASMOQUINE)

Diagrammatic representation of effects of causal prophylactics on pre-erythrocytic forms. Note that these drugs rapidly destroy the pre-erythrocytic or early a.s. forms. Blood smears fail to show parasites and subinoculations from the 7th day onwards prove that asexual parasites never appear in the blood.

In the second group seventeen *P. punctulatus punctulatus* harbouring sporozoites of *P. vivax* in their salivary glands engorged on five volunteers. Four of them received 80 mg plasmoquine (as base) daily on the day of exposure

and subsequent 8 days, and one received the same dose from the 9th to the 14th days (inclusive) after exposure. One volunteer who received plasmoquine from day 0 to +8 was subinoculated on the 11th, 15th and 43rd days after exposure. All these subinoculations were negative. During a period of 80 days' observation, no clinical or pathological evidence of vivax malaria was obtained (Table XI). The other four volunteers all developed overt vivax malaria while under observation.

These results indicated that plasmoquine base exerted an inhibitory effect on the schizogony of the early *ee* (pre-erythrocytic) forms since parasites were not demonstrable by subinoculation on the 9th and 10th day of the infection as normally invariably happens. In another instance parasites were not demonstrated by subinoculation on the 11th, 15th or 43rd day. Plasmoquine, however, did not destroy either the sporozoites or *ee* forms in at least four of the donors, since they later developed overt vivax malaria (Chart 13). The schizonticidal action of plasmoquine base even in this larger dosage of 80 mg daily was unsatisfactory.

The new 8-amino quinoline derivative—pentoquine—was not available until after the disbandment of the L H Q Medical Research Unit. In consequence, no work was done at Cairns on this drug.

## II THE BIGUANIDES

The brilliant researches of CURD, DAVEY and ROSE (1945) culminated in the synthesis of the biguanides M 4430 ( $N_1$ -p-chlorophenyl- $N_6$ -methyl- $N_6$ -isopropylbiguanide acetate) and M 4880 ( $N_1$ -p-chlorophenyl- $N_6$ -isopropylbiguanide acetate) now known as paludrine. Both these drugs were found to exert a causal prophylactic and therapeutic action in bird malaria. M 4430, however, proved to be a causal prophylactic against *P. gallinaceum* only, whereas paludrine was found to be a causal prophylactic against this species as well as against *P. cathemerium*, *P. lophurae* and *P. relictum*.

### (a) Plasmoquine (M 4888)

During the Cairns experiments detailed investigations were made on the action of this drug as a causal prophylactic, as a schizonticide and as a gametocide. Subinoculation proved of great assistance in interpreting the significance of some of the data obtained.

(1) *P. falciparum* infection.—In four of the five experiments (Table XII) twenty infected mosquitoes of the species *A. punctulatus punctulatus* containing viable sporozoites of *P. falciparum* in the salivary glands were allowed to engorge on each volunteer. In the other experiment nine to ten infective bites were given.

In the first experiment (Table XII) paludrine was administered 1 day before exposure, on the day of biting and for the subsequent 22 days. Subinoculations of 200 c.c. whole blood into six non-immune recipients were performed on the 7th day following exposure with negative results. The



donors never developed parasites or fever and no other evidence of malaria infection was ever noted. Controls included four volunteers taking atebryn (0.1 gramme daily), subinoculations on the 7th day in each instance yielded positive results, showing that malaria had been transmitted by the mosquitoes used in the experiments.

In the second experiment (Table XII) three volunteers received 300 mg of paludrine on the day before exposure, and for the following 6 days. Subinoculation from the control (200 c.c.) 6½ days after exposure was positive, the recipient developing typical overt falciparum malaria. Subinoculations (200 c.c.) done at the same time on the three volunteers taking paludrine were negative and they were ultimately proved to be cured.

A third experiment (Table XII) performed on three volunteers receiving only 100 mg daily for the same period yielded identical results. Subinoculations with 200 c.c. of blood transfused on the 7th day were negative and the donors as well as recipients failed to get malaria.

It was evident from these experiments that paludrine was acting as a causal prophylactic, providing a concentration of the drug was not persisting in the blood sufficient both to destroy the early asexual erythrocytic parasites when they appeared in the circulation and to render these parasites rapidly non-viable in subinoculated blood. It has been found by MAEGRAITH and his colleagues\* that paludrine given in a dosage of 100 mg daily disappears from the blood on the 3rd day. Under such circumstances 100 mg of paludrine could be given daily for 4 or possibly 5 days and the blood would contain little if any paludrine when subinoculations were performed 7 days later.

A fourth experiment (Table XII) designed to meet this requirement was performed. Twenty infective bites (*P. falciparum*) were given as in previous experiments. Four volunteers were given 100 mg of the drug on the day of exposure, in addition the first volunteer received 100 mg for the next 2 days (total = 300 mg), the second 100 mg for the next 3 (total = 400 mg), the third 100 mg for the next 4 (total = 500 mg), and the fourth 100 mg for the next 5 days (total = 600 mg). Subinoculation (200 c.c.) on the 7th day from the control who had received no drug produced typical overt malignant tertian malaria in the recipient, while subinoculations from all four volunteers taking paludrine were negative. Subsequent observation confirmed that all the original donors failed to develop malaria.

In another type of experiment where six volunteers were exposed to twenty infective bites the administration of paludrine was postponed until after subinoculation had been performed on the 7th day. Daily dosage (100 mg) was then commenced and continued for 14 days. The recipients all developed parasites (*P. falciparum*), and fever showing the donors had been infected, but the donors themselves failed to develop overt malaria being ultimately cured by the schizonticidal action of paludrine.

\* Private communication

As already reported by FAIRLEY and his colleagues (1946) it has also been found that if a single dose of 50 or 100 mg. of paludrine be given from 39 to 131 hours after exposure to infection malaria fails to develop. These and other experiments proved that paludrine acts as a true causal prophylactic in *P. falciparum* infection destroying the early a.c. (pre-erythrocytic) forms before the asexual erythrocytic parasites are liberated into the blood. (Chart 12, p. 668).

TABLE XII.

RESULTS OF SUBINOCULATIONS MADE ON THE 7TH DAY IN SIXTEEN VOLUNTEERS EXPOSED NINE TO TWENTY INFECTIVE BITES (*P. falciparum*) WHILE TAKING DAILY DOSES OF PALUDRINE.

Infective bites ( <i>P. falciparum</i> )	Numbers of volunteers in experiment.	Paludrine		Subinoculation.		Result.
		Dosage per day in mg.	Duration of administration in days.	Negative.	Positive.	
20	6	100	-1 to +23	0	0	Causal prophylaxis
20	3	300	-1 to + 6	3	0	
20	3	100	-1 to + 6	3	0	
10	1	100	0 to + 6	1	0	
9	1	100	0 to + 4	1	0	
10	1	100	0 to + 3	1	0	
9	1	100	0 to + 2	1	0	
9	1	100	0 to + 2	1	0	

It will be seen that in *P. falciparum* infections paludrine is (1) a causal prophylactic preventing asexual parasites appearing in the blood (2) a specific schizonticide causing both clinical and radical cure after asexual parasites have gained access to the circulating blood.

(2) *P. vivax* infection.—Five volunteers were bitten by twenty *A. punctulatus punctulatus* containing sporozoites in the salivary glands. Four of them received paludrine in a dosage of 300 mg. on the day before infection, on the day of infection and for the subsequent 7 days. The control volunteer subinoculated (200 c.c.) on the 9th day was positive the recipient developing a typical attack of overt benign tertian malaria. Subinoculations of the four volunteers taking paludrine were performed on the 9th day with negative results. Despite the fact that subinoculations were negative at this time each developed overt benign tertian malaria some 31 to 50 days after exposure to infection (Chart 13 p. 668).

Another group of four volunteers were similarly bitten by twenty *A. punctulatus punctulatus* containing viable sporozoites in their salivary glands. Each received 100 mg. of paludrine daily on the day before exposure to infection and for 23 days thereafter. Two subinoculations (200 c.c.) were made from each of the volunteers one on the 9th and one on the 13th day making total

of eight subinoculations. All were negative, none of the eight recipients developing malaria. Despite this fact, the four donors whose blood had twice yielded negative subinoculations all developed overt benign tertian malaria some 49 to 140 days after exposure to infection.

Four volunteers were exposed to twenty infective bites (*P vivax*). The first one received a single dose of 1.0 gramme of paludrine 3 hours before exposure. The second received 1.0 gramme 3 hours before exposure, this was repeated on the following day (total = 2 grammes). The third received 100 mg daily for the first 5 days and the fourth 100 mg daily for the first 6 days. Subinoculations on the 9th to 10th days were negative in each instance, yet overt vivax malaria developed 18, 31, 26 and 30 days respectively after exposure.

*Comment*—Though paludrine is an effective schizonticidal drug in vivax infections and inhibits the production of asexual erythrocytic parasites (micromerozoites) from the *ee* forms as revealed by subinoculation experiments, it acted only as a partial causal prophylactic in the dosage employed in these experiments (Chart 13). Though the incubation period was prolonged and negative subinoculations were found at a time when they would normally have been positive, radical cure was not attained with any of the therapeutic regimens adopted.

#### (b) M 4430 *P vivax* infection

While taking M 4430 in a dosage of 200 mg daily, six volunteers were bitten by twenty to twenty-two *A punctulatus punctulatus* containing viable sporozoites of *P vivax* (New Guinea strains) in their salivary glands. During the period of drug administration, they were protected against overt malaria and parasites were not demonstrated in thick blood films. Early premonitory symptoms of suppressed malaria were entirely absent, though present in a control volunteer infected by the same batch of mosquitoes while taking atabrin, 0.1 gramme daily.

Subinoculations were made from all six volunteers on the 9th day after exposure to infection, these were uniformly negative. None of the recipients of 200 c.c. of their blood subsequently developing either demonstrable parasites or overt malaria. Despite these findings, all the six donors originally exposed to infective biting developed overt benign tertian malaria on an average of 32.5 days after ceasing the administration of M 4430.

*Comment*—The results of these subinoculation experiments indicate that M 4430 prevented the normal liberation of erythrocytic parasites (micromerozoites) from pre-erythrocytic or early *ee* forms on the 9th day. It failed, however, to eradicate completely the persistent *ee* forms (phanerozoites) when administered in a dosage of 200 mg daily for 23 days after inoculation of sporozoites since overt malaria invariably developed later. It follows that M 4430 is a partial causal prophylactic in vivax infections (New Guinea strains) and has an action in this respect which is similar to, or identical with, that of paludrine (M 4888) and plasmoquine.



## SECTION III.

## SUMMARY AND CONCLUSIONS

In this paper the value of subinoculation in the study of human malaria has been extended and a new technique developed consisting of the transfusion of larger quantities of blood (200 c.c.) by the Juhani Smith direct transfusion apparatus from the malaria-infected donor into the non-immune recipient. Special attention has been directed to determine the distribution of plasmodia in the blood at different periods after inoculation of sporozoites by infective mosquitoes the action of anti malaria drugs on different stages of the life cycle of malaria parasites, and indirect evidence regarding the existence of an exo-erythrocytic cycle in man.

1. Four out of the following six phases in the life history of malaria parasites in man have been demarcated by subinoculation during these investigations.

(a) The initial invasion stage when viable sporozoites may be demonstrated in the circulating blood for short periods ( $\frac{1}{2}$  to 1 hour) after inoculation of sporozoites by anopheline mosquitoes into the tissues or directly into the blood vessels.

(b) The negative blood phase or prepatent period when pre-erythrocytic or early e.c. forms are presumably undergoing schizogony in reticulo-endothelial cells this lasts approximately 6 days in *P. falciparum* and 8 days in *P. vivax* during which time massive subinoculations of blood from heavily infected volunteers uniformly fail to induce malaria in recipients.

(c) The initial stage of parasitaemia which is generally of submicroscopic density. Here there is an invasion of the blood by first generation merozoites (micromerozoites of REICHNOW and MUDROW) presumably liberated from the pre-erythrocytic or early exo-erythrocytic forms. This occurs on the 7th day (144+ hours) in *P. falciparum* and on the 9th day (192+ hours) in *P. vivax*. The first generation of erythrocytic parasites are usually of submicroscopic density being rarely revealed even after prolonged examination of thick blood films. Their presence is shown by positive subinoculations and it is generally about 3 days before parasites are demonstrated microscopically. This stage may be manifest clinically by minor premonitory clinical features such as headache, backache, generalized muscular pains and tenderness over the liver. Haematologically there is a characteristic left polymorphonuclear shift, a relative leucopenia and a decrease in lymphocytes.

(d) Overt malaria, associated with primary fever major clinical features and demonstrable parasites in blood films. In *falciparum* malaria parasites are first demonstrable in thick films (1 per c.mm.) on an average of 9.5 days after a single exposure (range 7 to 12 days). Trophozoite densities increase in phase rapidly there being a big rise each 48 hours followed by a smaller fall. In untreated heavy infections clinical evidence of hyperinfection is manifest about the 17th to 18th day unless treatment is commenced not later than the 19th



attacks were absent but the symptoms included headache, malaise, anorexia, slight transient elevations of temperature (99° F. to 100° F.), splenomegaly, hepatomegaly (one case), left neutrophil shift and some fall in haemoglobin and red blood cell counts. Though the blood was frequently and thoroughly searched for parasites they were never demonstrable microscopically. Subinoculations performed on the 53rd day following falciparum infection and the 67th day following the vivax infection were negative. They were not done earlier and for this reason the presence of parasites in submicroscopic densities in the early phases of infection cannot be excluded in either instance with certainty. In addition to the small number of sporozoites injected the hypothesis suggested to explain the syndrome is that the sporozoites become modified in some manner by transfusion so that either exo-erythrocytic schizogony did not proceed beyond the macrogamont and macromerogont stages described by REICHNOW and MUNOW (1943) in *P. praecox* or if it did, so few micromeronts were produced or they were so dentalized that they never attained a density which was demonstrable microscopically. Under the latter circumstances successive invasion of endothelial cells might be maintained but not successive invasion of red corpuscles with establishment of the asexual erythrocytic cycle and overt attacks.

3 Subinoculation experiments in experimentally infected volunteers taking adequate daily doses of anti malaria drugs and failing to develop overt malaria, have afforded most valuable information regarding the mode of action of these drugs as suppressants or causal prophylactics.

*Negative subinoculations.*—Under such circumstances in *P. falciparum* infection a negative subinoculation on the 7th day indicates that the drug is acting as a complete causal prophylactic. Paludrine and plasmoquine both fall into this category. Since subinoculations remain constantly negative, and overt attacks do not occur it is evident that viable parasites never gain access to the blood. In *P. vivax* infection a negative subinoculation on the 9th day indicates that the drug has either destroyed the sporozoite or the early c.c. forms or temporarily inhibited their development, i.e. the drug is a complete or partial causal prophylactic. Paludrine and plasmoquine both proved to be partial causal prophylactics since, despite negative subinoculations, the donors finally developed frank clinical malaria with parasites (*P. vivax*) in the blood smear.

*Positive subinoculations.*—A positive subinoculation on the 7th day in *P. falciparum* infection and on the 9th day in *P. vivax* infection indicates that the drug is neither a complete nor a partial causal prophylactic, and if the experimentally infected volunteer subsequently fails to get an overt attack while taking the drug it implies that the drug is suppressing malaria by schizonticidal action.

When schizonticidal drugs of atebnin type are given in adequate dosage each day for suppressive purposes positive subinoculations are obtained with 200 c.c. blood on the 7th day in *P. falciparum* and on the 9th day in *P. vivax*.

with remarkable constancy, just as if no anti-malarial drug was administered. Despite the fact that parasites are not being found in blood smears they may be demonstrable by subinoculation for the next 3 to 5 days but not subsequently. While a negative subinoculation from the 13th day onwards indicates radical cure in *P. falciparum* malaria, it generally only indicates suppression in *P. vivax* infections. The daily dosage of the schizonticidal drugs so tested were atebirin (0.1 gramme), sontochin (0.1 gramme), resochin (0.1 gramme), quinine (grains 20), sulphadiazine (1.0 gramme). Sulphadiazine proved an effective suppressant and radically cured *P. falciparum* infections, but failed to suppress most *P. vivax* infections. The others suppressed B T, and suppressed and cured M T infections.

4 No significant difference in the suppressive and curative value of atebirin was noted in sporozoite-induced or trophozoite-induced falciparum malaria. Absence of overt attacks, late negative subinoculations and absence of pre-munity as judged by susceptibility to re-infection characterized both groups of experimentally infected volunteers indicating that radical cure had been achieved. Since data on subinoculation indicate that in falciparum malaria once parasites are completely eradicated from the blood the infection is radically cured, and as radical cure in sporozoite-induced and trophozoite-induced falciparum malaria are equally readily attained, it would appear that the *ee* forms of *P. falciparum* do not persist beyond the pre-erythrocytic stage—presumably because they are completely converted into first generation merozoites (micromerozoites). There is no evidence in man that late *ee* forms are produced from asexual forms.

5 A very remarkable difference was found in response to treatment with atebirin between sporozoite-transmitted and trophozoite-transmitted vivax malaria. While trophozoite-induced malaria was radically cured by atebirin in a suppressive dosage (nine out of nine) or therapeutic dosage (eighty-eight out of ninety), radical cure in sporozoite-transmitted vivax malaria was difficult to attain. Not more than 3 per cent. of volunteers on suppressive atebirin and not more than 18 per cent. receiving one course of Q A P treatment for vivax malaria were radically cured. The facility with which the parasites are permanently eradicated from the blood in trophozoite-transmitted vivax malaria and the high frequency of relapse in treated sporozoite-induced infections support the view that the different therapeutic response is due to the persistence of late *ee* forms which are more resistant to treatment and which after a variable period liberate into the blood stream parasites (micromerozoites) giving rise to vivax recrudescences, relapses and recurrences. There is no evidence that asexual parasites give rise to late *ee* forms in vivax infections.

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# MONKEYS IN RELATION TO YELLOW FEVER IN BWAMBA COUNTY, UGANDA

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During the past 6 years observations on the epidemiology of yellow fever have been carried out in Bwamba County, Uganda. Bwamba is a small, heavily forested area lying between the north spur of the Ruwenzori Mountains (Mountains of the Moon) and the Semliki and Lamia Rivers, which form part of the Uganda-Congo boundary. The topography and vegetation have been described in detail elsewhere (HADDOW, 1944), but it is necessary here to include a brief account.

The main part of the county lies in the Western Rift Valley, at an altitude of 2,500 to 3,500 feet. The north-eastern part of this lowland country consists of the open Semliki Plains, which stretch away to the shores of Lake Albert. These grasslands are apparently not of importance in the local epidemiology of the disease and need not be mentioned further. The northern half of the remaining lowland country is occupied by the Semliki Forest, an eastward extension of the great Ituri Forest of the Congo (Fig 1). The Semliki Forest has been designated a sleeping-sickness area and is closed to human occupation. It contains large tracts of swamp and high bush, but in the main consists of dense primary rain-forest. The fauna is essentially "West African" and is

\* During the first year of the monkey investigation most of the specimens were collected by our field assistant, Mr J MASCARENHAS, who conducted his work with admirable persistence under difficult and tiring forest conditions. Colonel C R S PITMAN, Game Warden, Uganda Protectorate, and Mr G H E HOPKINS, Senior Entomologist (Medical), Uganda Protectorate, have helped us in many ways and have shown a constant interest in the progress of the investigation.

† This Institute is supported jointly by the Medical Department of the Uganda Protectorate and the International Health Division of The Rockefeller Foundation.

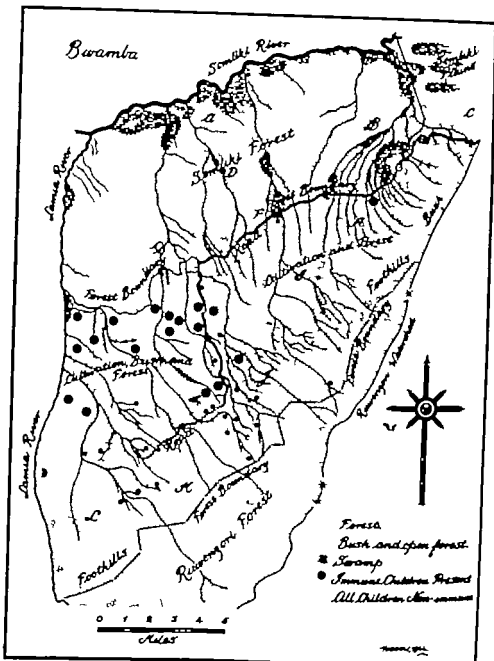


FIG. 1.—Sketch map of Bwamba County to show the distribution of forest and its relationship to yellow fever immunity among children (The distribution of child immunity is based on work carried out before the mass-vaccination campaign.)

typical of the zoogeographical area known as the Lower Guinea Forest District. Big game abounds in the Semliki Forest. The monkey population also is exceptionally large and varied. Between the forest and the Ruwenzori foothills lies a rolling stretch of country, densely populated and heavily cultivated, but intersected by many relict strips of primeval forest in the deep gorges of the rivers which flow from the mountains. The foothills of the mountain below the boundary of the Ruwenzori Forest (at about 7,000 feet) consist mainly of open grasslands with scattered areas of cultivation, but narrow belts of gallery forest occur in the many small river valleys. Above 7,000 feet lies a tract of dense cloud-forest, which gives way, at about 8,000 feet, to a zone of mountain bamboo. At 9,000 feet the bamboo is replaced by an alpine flora extending upward to the bare rock of the high peaks and ridges, which in one part of Bwamba attain a height of over 14,500 feet. The climate of the Bwamba lowlands is warm and very humid. The rainfall, of some 50 to 60 inches per year, is well distributed, and there is no dry season in the ordinary sense. On the mountain slopes mist and rain prevail throughout a great part of the year and marked extremes of temperature occur. No precise data concerning rainfall on the mountain are available, but it is known to be extremely high.

In 1937 immunity to yellow fever was discovered among the inhabitants of Bwamba, and intensive survey work was begun (MAHAFFY *et al*, 1942). This revealed that, while immunity among adults was common in many parts of the country, the percentage of immunes rose steadily as the edge of the uninhabited Semliki Forest was approached, and immunity in children was largely restricted to areas adjoining the forest boundary. The survey also showed (Fig 1) that the mountain slopes were not of importance in the local epidemiology (HUGHES *et al*, 1941). Intensive work in the area of high immunity culminated in the isolation, in 1941, of yellow fever virus from a human patient and from wild-caught mosquitoes—*Aedes (Stegomyia) simpsoni* Theo (MAHAFFY *et al*, 1942). Following these discoveries, very complete mass vaccination of the entire population of Bwamba was carried out, with the intention of preventing possible eastward spread of the disease from what was evidently an endemic focus. After the vaccination campaign an extensive programme of mosquito study was begun in Bwamba and is still in progress. Yellow fever virus was again isolated from *A. simpsoni* in June, 1942, and from forest-dwelling *Aedes* spp (captured in uninhabited rain-forest) in April, 1944. As it is known that over 90 per cent of the entire population of Bwamba is still immune to yellow fever as a result of vaccination (SMITHBURN and MAHAFFY, 1945), these isolations imply the persistence of the disease among the wild animals of the Semliki Forest.

During the survey period prior to the first isolation of virus, five redtail monkeys (*Cercopithecus nictitans mpangae* Matschie) from unrecorded Bwamba localities were tested for immunity by the protection test. As one of these proved to be immune, it was decided that further observations on animals in



relation to yellow fever were desirable and these have been carried on concurrently with the mosquito work. Though many groups of animals have been investigated, immunity to yellow fever has so far been found among monkeys only. The specimens have consisted mainly of blood samples collected in the field, but over twenty five monkeys have also been obtained for protection test and experimental work. The purpose of this paper is to discuss the results of tests on the 150 Bwamba monkeys studied between March, 1942, and November 1944.

### LABORATORY TECHNIQUE.

The term immunity as used in this report refers to the presence in the serum of specific protective antibody against yellow fever virus. The presence or absence of such antibody was determined by intraperitoneal mouse protection tests, made in mice either 5 to 6 weeks old or 14 days old. Both methods of testing employed 1 per cent. suspensions of mouse brain passage neurotropic virus made up in 10 per cent. non immune serum in physiological saline. Briefly the techniques were as follows:

1. Adult mice 35 to 42 days old, received a preparatory intracerebral injection of 0.03 ml. each of sterile 2 per cent. starch solution. This was followed in 10 to 30 minutes by the intraperitoneal inoculation of 0.6 ml. per mouse of the serum virus mixture made by adding 1.5 ml. of 1 per cent. virus to 3.0 ml. of serum. Six mice were used for each such test.

2. Mice 14 days old, in litters usually accompanied by mothers, were inoculated intraperitoneally with 0.06 ml. each of serum-virus mixture made by adding 0.25 ml. of 1 per cent. virus suspension to 0.5 ml. of serum. Two litters of four or five mice each were used for testing each serum. These infant mice received no preparatory inoculation of starch solution, and anaesthesia was not necessary for the intraperitoneal injection.

All mice were observed for 10 or 12 days. Sera were considered protective if not more than one mouse in a group died. Details of methods and interpretation of protection test results are given elsewhere (SATHIAPAN 1945). The data in this report include tests on 150 sera. Of these sixty nine were tested once, seventy three were tested twice, five were tested three times, and three were tested four times: the total number of tests on the 150 sera being 242. Of the sixty nine sera tested once, twenty-eight were non-protective and forty-one protective. One serum gave an unsatisfactory result on first test and was protective on retest; two gave inconclusive reactions in the first tests and were non-protective on retest; two others gave inconclusive reactions on first test and were protective on retest. Twenty-eight sera gave repeated non-protective results and forty-eight gave repeated protective results. Discordant results were not encountered.

## FIELD METHODS

In procuring monkeys in the field for bleeding we have found a double-barrelled 12-bore shotgun the most useful weapon, though we have also made much use of a medium-calibre (0 303) rifle, particularly when hunting large species such as baboons or colobus. In the case of the shotgun we have found it a useful practice to load the choke barrel with heavy (SSG) shot for man-gabeyes and colobus, and the other with a lighter shot (No 2, 3 or 4) for smaller monkeys.

If a satisfactory specimen is to be obtained, it is necessary to recover the body very quickly—often a matter of considerable difficulty in dense bush or forest. If more than 10 minutes elapse before recovery of the body, the blood will probably have begun to clot and will choke the fine hypodermic needle of the venule. Clotting is particularly rapid in the case of monkeys shot through the chest. Whenever possible, the specimen should be obtained while the heart is still beating (it may continue to do so for as much as 15 minutes after the animal becomes prostrate). The necessity for rapid recovery of the body leads us to prefer soft-nosed or dum-dum bullets as a monkey drilled by a hard-nosed bullet may pass through several trees before falling, even when very seriously wounded. This, of course, adds to the difficulty of securing the specimen. The soft-nosed bullet or dum-dum is also a humane measure, as a wounded monkey which escapes will almost certainly bleed to death rapidly.

When the body is obtained, it is laid back-downward, an assistant holding the arms spread wide apart. The skin is slashed open from throat to pubis with a curved skinning-knife and an incision is made opening the abdominal cavity. The chest is then opened with a large pair of plaster-shears (the lower blade blunt-pointed to avoid injury to internal organs), the costal cartilages being severed along the left sternal margin. The diaphragm is now cut round its right margin and the pericardium is picked up with forceps and opened carefully with a pair of scissors. The tip of the ventricles is picked up with rat-tooth forceps and the specimen is taken from one of the auricles—usually the right. It is seldom that a satisfactory specimen can be obtained from the ventricles, even when the heart is beating quite strongly. In cases where the heart does not yield a satisfactory specimen, it is often possible to obtain blood in abundance from the great vessels—the superior or inferior vena cava, or the pulmonary, renal, or iliac vessels. It is for this reason that at the beginning of the operation the abdomen is opened and the diaphragm severed from its origin, for after failure to obtain blood from the heart little time remains for bleeding and these measures enable the large veins to be displayed at once.

Whenever possible several specimens are taken, in case one may be contaminated. This is particularly desirable, of course, when the viscera have been torn by the shot. The specimens are labelled and are at once transferred to a thermos flask filled with ice. They are then kept continuously under

refrigeration until the test is performed. In cases where no specimen can be obtained from the heart or vessels it is always worth while to take up some loose blood from the chest or abdomen. Where refrigeration is available such specimens will often allow a successful test to be made on the blood of an animal which would otherwise have been wasted.

After collecting the blood sample, we record detailed data, including temperature of the animal scientific and popular names sex, estimated age, state of teeth, pelage, and genitalia weight and measurements, mouth and cheek pouch contents, nature and weight of stomach contents, parasites, and abnormalities, activities when first seen, specimens taken, number date, collector time altitude type of country weather etc.

The field kit at present used in Bwamba comprises

1 machete.	1 roll $\frac{1}{2}$ -inch adhesive tape (for labelling venules).
curved skinning knives.	
1 pair plaster shears (lower blade blunt pointed)	1 thermos flask (quart or $\frac{1}{2}$ -gallon size) filled with ice cubes.
1 pair 4-inch rat tooth forceps.	1 clinical or veterinary thermometer.
1 pair pointed scissors, medium size.	1 spring balance (weighing to 50 lb.).
25 crules (30 ml. capacity).	1 2-metre spring-steel tape measure (graduated in mm.)
1 box glass files	Pencil and field-book.

With the exception of the machete and thermos flask, which are carried separately the entire kit fits easily into a light wooden box with sides 10 inches square

#### THE PRIMATES OF BWAMBA.

Twelve species and subspecies of Primates have so far been found by us to occur in Bwamba. It is necessary at this point to give some account of their habits and distribution, as these have proved to be of importance in relation to yellow fever

Apart from monkeys, two species belonging to the Lemnuroidea have been obtained, the small forest galago or "bush baby" (*Galago demidoffi thomasi* Elliot) and the potto or African slow lemur (*Perodicticus potto ibeatus* Thomas). The forest galago is delicate in captivity and none of the few immature specimens so far obtained has survived for protection test. Two pottos from Bwamba have been tested. Both were non-immune

The various Anthropoidea may now be discussed.

#### 1 *Cercopithecus albigena johannstoni* Lydekker (Black Mangabey)

The black mangabey is confined mainly to swampy areas of low-lying mixed forest where oil palms are abundant. One of its native names means the oil-palm thing. Thus extremely agile and wary monkey occurs in bands

Since writing this paper we have obtained specimens of *Cercopithecus neglectus* Schlegel and *Colobus badius* *eliasi* Dollman (both in the Semliki Forest, Fig. 1 area E), thus bringing the total number to fourteen.

of twenty to forty. It is very difficult to approach, and specimens have been hard to obtain. It is strictly arboreal and rarely descends to the ground except when alarmed or hunted, though it occasionally feeds in low forest vegetation only a few feet above the ground. The food consists mainly of oil-palm nuts, though other fruits and green shoots have sometimes been found in the stomach. The black mangabey is a monkey of the uninhabited forest and rarely occurs in the proximity of dwellings. In Bwamba it is commonest in the north-eastern forest (Fig 1, area E) and in the palm and acacia belt along the banks of the Semliki River. J. A. ALLEN (1925) notes that it sometimes wraps its tail round a branch for support—perhaps an indication of future prehensile tendencies—and that the tails of adults are generally somewhat threadbare in consequence. We are able to confirm this observation.

## 2 *Cercopithecus aethiops centralis* Neumann (Grey Monkey)

This species, which spends much of its time on the ground and in low bush and second growth, has a very limited distribution in Bwamba. It is confined to the open grass and bush areas (Fig 1, areas C, K and L). It also occurs in the palm belt along the Congo shore of the Semliki River.

## 3 *Cercopithecus l'hoesti l'hoesti* Sclater (l'Hoeest's Monkey)

This rare and little-known monkey is restricted to the lower border of the Ruwenzori Forest and to the high forested valleys at altitudes of 6,000 to 8,000 feet. It is essentially a ground species, which takes to the trees unwillingly when hunted by dogs. It occurs in large bands which frequently plunder banana plantations on the mountain.

## 4 *Cercopithecus mitis stuhlmanni* Matschie (Blue Monkey)

In Bwamba this beautiful monkey is confined mainly to the Ruwenzori Forest and to the forested areas below the foothills (Fig 1, areas H and I). It sometimes descends in large bands to the north-eastern part of the Semliki Forest (Fig 1, area E). These periodic visits occur mainly during the rains when fruits, which form a large part of its diet, are particularly abundant in this part of the forest. In addition to fruits, the blue monkey is especially fond of insect food. The Bakonjo tribe of Ruwenzori report that it frequently raids banana plantations and maize fields below the mountain forest boundary, but in its periodic descents to the lowland forest it is not a crop-raider, as the area it visits is uninhabited. The blue monkey is essentially arboreal, but descends to the ground when alarmed by shooting or by the presence of its enemy, the monkey-eating eagle (*Stephanoaëtus coronatus*).

## 5 *Cercopithecus mona denti* Thomas (Dent's Monkey)

This rare monkey is confined to the lowland forest (Fig 1, areas D, E, F and G). It is very shy and seldom seen. It occurs in large bands, but, on

account of its elusive nature nothing is known of its habits except that it is mainly arboreal. The stomach of one of these monkeys has been examined, and the contents were found to consist entirely of leaves and shoots, without fruits or berries of any kind. Our four specimens are the first recorded from Uganda.

6 *Cercopithecus nictitans mpanzes* Matschie. (Redtail Monkey)

This bold and very common species occurs in large bands, and is most prevalent round the edges of the lowland rain forest (Fig 2). Though mainly arboreal, it often descends to the ground when hunted, or to cross an open space. The redtail is a notorious raider of crops chiefly maize and bananas, but in the uninhabited forest its food consists mainly of oil-palm nuts and other tree fruits and berries. It is worth noting that these monkeys return nightly to a small sleeping area, though on different nights they may rest in different trees within its limits. In the case of one band, such an area is known to have been used in this way for the past 18 months.

7 *Papio doguera temelatus* Elliot. (Anubis Baboon.)

Baboons in Bwamba occur mainly on the mountain slopes at altitudes of 4,000 to 6,000 feet, but a band of about sixty inhabits the north-eastern corner of the Semliki Forest (Fig 1 area E). Though essentially ground monkeys, baboons spend a considerable time feeding in trees. In some areas they also sleep in trees, at heights of as much as 100 feet above ground. Their food consists mainly of oil-palm nuts, fruits, insects and millipedes, but they are of course notorious crop-raiders. The local bands occupy well-defined territories beyond the limits of which they seldom wander. The presence of occasional albinoid individuals aids in the identification of a given band, and one particularly pale yellow female was seen at regular intervals in one small area over a period of about 5 years.

8 *Colobus polykomos wellensis* Matschie (Lowland Colobus.)

This is the commonest of the Bwamba monkeys, with the possible exception of the redtail. It inhabits the denser areas of the lowland rain-forest, and also occurs in the forested areas below the Ruwenzori foothills (Fig 3). The bands usually consist of ten to twenty individuals, but solitary males are frequently encountered. The lowland colobus is a strictly arboreal species, which rarely leaves the trees except when alarmed by a shot or when attacked by *Stephanoxenus*. The food consists almost entirely of leaves and young green shoots, the tree of preference in Bwamba being the African ironwood (*Cynometra alexandri*). This monkey does not raid crops, and in general it avoids the vicinity of dwellings, being scarce in all the populated areas. Like the redtail the lowland colobus has the habit of returning nightly to the same small sleeping area.

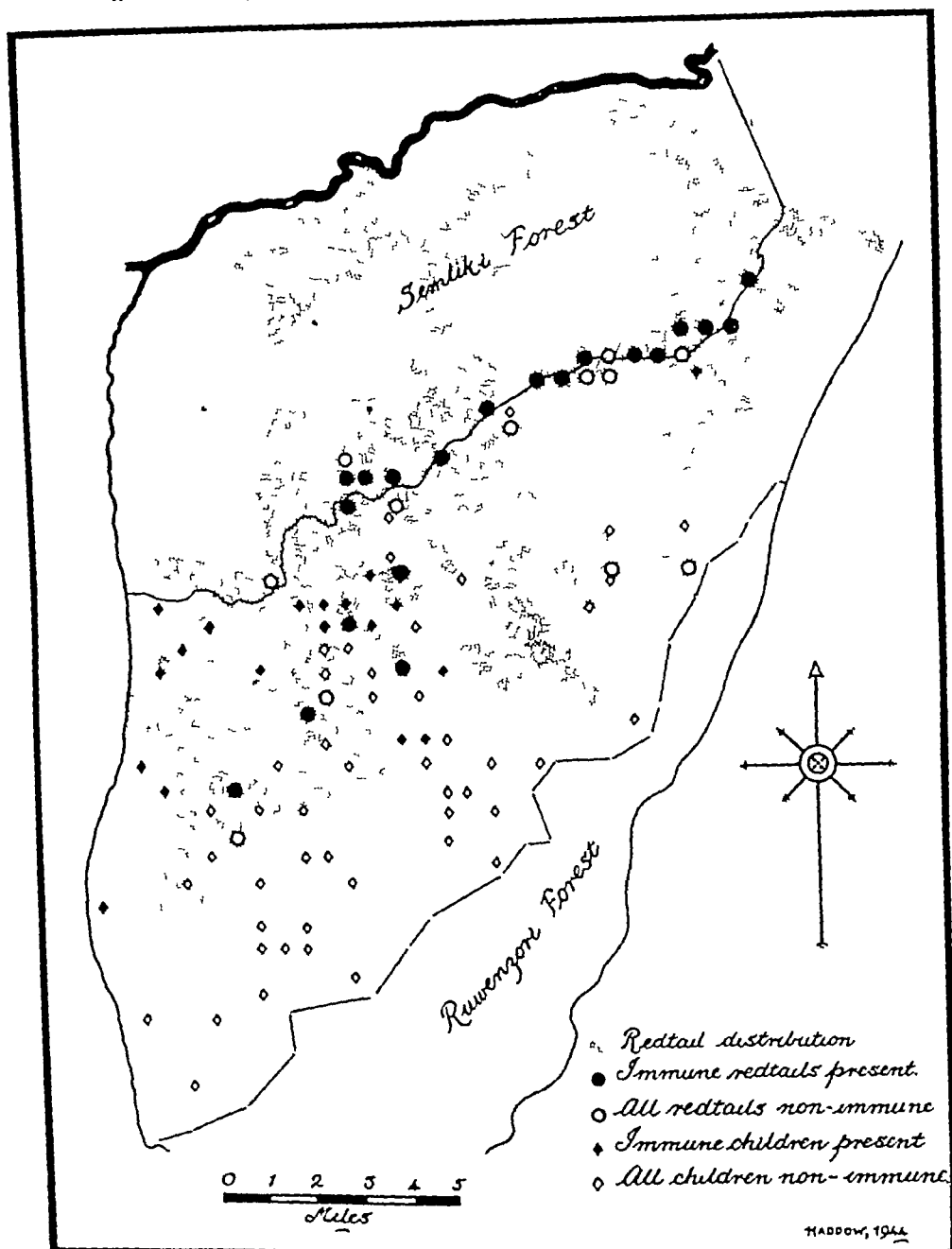


FIG 2—Sketch map of Bwamba County to show the distribution of immunity to yellow fever in the redtail monkey and the known range of this species in relationship to the distribution of yellow fever immunity among children (data concerning children as in FIG 1)

For purposes of this map the country has been divided into areas each  $\frac{1}{4}$  mile square, all tests in each such area being treated collectively. Thus a single circle or diamond may represent many tests

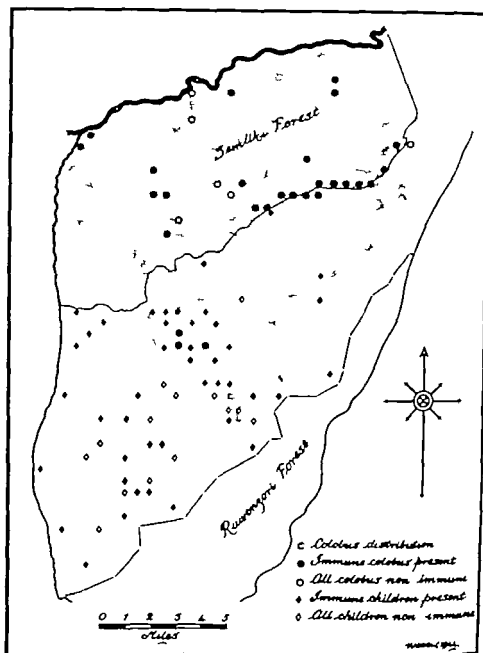


FIG. 3.—Sketch map of Bwanibwa County to show the distribution of immunity to yellow fever in the lowland colobus monkey and the known range of this species in relationship to the distribution of yellow fever immunity among children. Details as in legend FIG. 2.





TABLE I.

IMMUNITY TO YELLOW FEVER AMONG SWAMRA MONKEYS. RESULTS OF PROTECTION TESTS.

Species	Age grade.	Male.			Female.			Both sexes.		
		Im- mune	Non- im- mune	T o t a l	Im- mune	Non- im- mune	T o t a l	Im- mune	Non- im- mune	Total
Black mangabey	Subadult	1	0	1	0	1	1	1	1	2
	Adult	3	0	3	1	1	2	4	1	5
	Old	—	—	—	3	0	3	3	0	3
	Subtotal	4	0	4	4	2	6	8	2	10
Grey monkey	Juvenile	0	1	1	—	—	—	0	1	1
	Subtotal	0	1	1	—	—	—	0	1	1
Flourent monkey	Juvenile	—	—	—	0	1	1	0	1	1
	Subadult	—	—	—	0	1	1	0	1	1
	Subtotal	—	—	—	0	2	2	0	2	2
Blue monkey	Juvenile	—	—	—	0	2	2	0	2	2
	Subadult	0	1	1	—	—	—	0	1	1
	Adult	2	1	3	1	0	1	3	1	4
	Subtotal	2	2	4	1	2	3	3	3	6
Dent's monkey	Subadult	0	1	1	1	1	2	1	2	3
	Adult	—	—	—	1	0	1	1	0	1
	Subtotal	0	1	1	2	1	3	2	2	4
Redtail	Juvenile	1	0	1	0	2	2	1	0	1
	Subadult	2	2	4	2	1	3	4	3	7
	Adult	16	6	22	6	1	7	22	7	29
	Old	2	0	2	1	0	1	3	0	3
	Subtotal	21	8	29	9	3	12	29	10	39
Baboon	Juvenile	0	2	2	0	1	1	0	3	3
	Subadult	0	1	1	—	—	—	0	1	1
	Adult	1	1	2	1	0	1	2	1	3
	Old	2	0	2	—	—	—	2	0	2
	Subtotal	3	3	6	1	1	2	4	2	6
Lowland colobus	Juvenile	0	2	2	0	2	2	0	2	2
	Subadult	0	2	2	1	2	3	1	4	5
	Adult	10	2	12	12	2	14	22	4	26
	Old	0	0	0	4	0	4	12	0	12
	Subtotal	10	4	14	17	4	21	35	6	41
Chimpanzee	Subadult	0	1	1	—	—	—	0	1	1
	Subtotal	0	1	1	—	—	—	0	1	1
All species	Juvenile	1	2	3	0	3	3	1	3	4
	Subadult	2	3	5	3	3	6	5	5	10
	Adult	19	3	22	19	3	22	41	8	49
	Old	2	0	2	5	0	5	14	0	14
	Total	24	8	32	27	9	36	61	16	77



Mosquito catches made in treetops in Bwamba have shown that here also the species of mosquitoes principally suspect of being involved in the transmission of animal yellow fever in the forest are mainly arboreal. Consequently it was at first thought that the more arboreal species of monkeys might show a higher incidence of immunity to yellow fever than those which spend much of their time on the ground. While the crude figures seem to support this hypothesis, the correction of frequencies and rates to a standard population reduces the differences between the groups of partly terrestrial mainly arboreal, and arboreal monkeys to a level which the  $\chi^2$  test shows to be not significant, there being a probability of 0.48 (or about 1 in 2) that the divergencies could arise by chance (Table III).

TABLE III.

IMMUNITY AMONG BWAMBA MONKEYS WITH RESPECT TO HABITAT CORRECTED TO STANDARD POPULATION.

Habitat.	Species.	Number.			Per cent. immune.	
		Immune	Non immune.	Total	Uncorrected.	Corrected.
Partly terrestrial	Grey monkey Howler's monkey Baboon	7	7	14	79	80
Mainly arboreal	Chimpanzee Blue monkey Dart monkey Redtail	40	20	60	88	87
Arboreal	Black mangabey Lowland colobus	45	31	76	70	80
Totals	Nine species	92	58	150	81	81

$$\chi^2 = 1.433 \quad n = 2 \quad P = 0.48.$$

While this finding implies a roughly equal exposure to infection in the case of all habitat groups of Bwamba monkeys it still remains almost certain that transmission must occur mainly in the trees for certain species which rarely descend to the ground (the lowland colobus and black mangabey) show a very high incidence of immunity to yellow fever. It follows that the vector probably is most active at a time when all monkeys are in the trees, i.e., at night. Field observation in Bwamba has shown that all monkeys, including the partly terrestrial baboon take up their sleeping posts in the trees just after sunset—about a quarter of an hour before dusk.

Among the arboreal mosquitoes of the Semliki Forest the majority bite by day, but one species, *Aedes (Stegomyia) africanus* Theobald, shows a very pronounced peak of biting activity in the hour after dusk, when all monkeys are in the trees and quiescent (HADDOW *et al.*, 1947). When it is added that this mosquito has been shown capable of transmitting yellow fever in the laboratory (PHILIP, 1929) and that in Bwamba it is the most abundant culicine at levels of 50 to 80 feet above ground, there seems every reason to suspect that it may play a large part in the local epidemiology of monkey yellow fever.

#### IMMUNITY IN RELATION TO LOCALITY AND DISTRIBUTION

Field observation leads us to believe that monkeys wander much less than is popularly supposed. Each band has its own territory, within the limits of which it may be seen day after day traversing the same treetop paths and nightly returning to the same sleeping area. Further, the fact that certain species such as l'Hoeest's monkey and the highland colobus are restricted to very small areas indicates that in such cases any tendency to wander is checked by an environmental barrier. It is interesting to note that GILMORE (1943) arrived at similar conclusions as the result of trapping and marking experiments in South America. His work showed, among other things, that a monkey transported from its native area might return there, though in a few cases evidence of wandering by marked monkeys was obtained. ZUCKERMAN (1932) also quotes various opinions which lead to the belief that monkeys of a given band tend to be restricted to a small territory. These observations are obviously of epidemiological significance.

Within the Bwamba lowlands there is little variation in the incidence of immunity between one district and another. Immune monkeys occur in most areas, both in the inhabited zone and in the closed forest. On the other hand, the eleven monkeys so far taken on the slopes of the Ruwenzori foothills have all been non-immune. This absence of immunity cannot be taken as applying to the whole monkey population of the mountain, as all the specimens concerned have been juvenile or subadult, age grades in which immunity is low in all areas. The finding is very suggestive, however, when taken in conjunction with the total absence of child immunity in the same region.

In Fig. 2 is shown the distribution of the redtail monkey. It will be seen that this species is common in the inhabited areas and that immune specimens have been taken far outside the boundaries of the Semliki Forest, often in localities where immunity among children has also been discovered. It seems very probable that during its frequent raids on banana plantations and maize fields it may pass on yellow fever virus to *Aedes simpsoni*, which abounds in such habitats and which is obviously the main vector of human yellow fever in Bwamba. In the present state of knowledge we regard the redtail as forming a link between the animal-to-animal cycle of the uninhabited Semliki Forest and the human disease of the populated areas.

Fig 3 shows the distribution of the lowland colobus, a true forest species which avoids the vicinity of dwellings. It will be noted that immune specimens have been obtained in many parts of the Semliki Forest, sometimes as much as 6 or 7 miles from the nearest habitations. The high incidence of immunity in this species (59 per cent. immune, corrected rate) combined with its abundance and wide distribution in the forest, leads us to the conclusion that in Bwamba it is probably to be regarded as the main animal host involved in the non-human yellow fever cycle. We do not suggest that it is in any way a reservoir of yellow fever for it seems likely that in Bwamba as in Colombia (Bugner *et al.* 1944) the insect vector rather than the mammalian host is to be regarded as the virus reservoir.

In Fig 4 is shown the distribution of the minor species. From this map it will be seen that while immunes do occur among these monkeys, they have been obtained mainly in areas which are uninhabited or at most sparsely populated by man. The absence of immunity among specimens taken on the mountain, coupled with the total absence of immunity among children in this area is again pointed out as probably significant. The monkeys of this group are all too scarce or too local to be of importance, with the possible exception of the black mangabey. The high incidence of immunity among mangabeys (corrected rate 60 per cent.) indicates that it must play a part in the monkey-to-monkey cycle, particularly in the palm areas where the lowland colobus is scarce or absent. On the other hand, its restricted distribution and relative scarcity imply that it cannot fill such an important role as the widespread and abundant colobus, while its wariness and strict avoidance of areas inhabited by man indicate that it probably has no direct relationship to the human disease.

Our conclusions are thus that the lowland colobus is probably the main animal involved in the yellow fever cycle of the uninhabited forest, that the redtail may play an important part in bringing virus into contact with man, and that the remaining species—on account of restricted habitat, scarcity or retiring habits—are of much less importance in the epidemiology of yellow fever in Bwamba.

TABLE IV  
IMMUNITY AMONG BWAAMBA MONKEYS WITH RESPECT TO  
SEX, CORRECTED TO STANDARD POPULATION.

Result.	Male	Female.	Total
Immune	56	36	92
Non-immune	37	21	58
Total	93	57	150

$$\chi^2 = 0.1194 \quad = 1 \quad P = 0.72$$

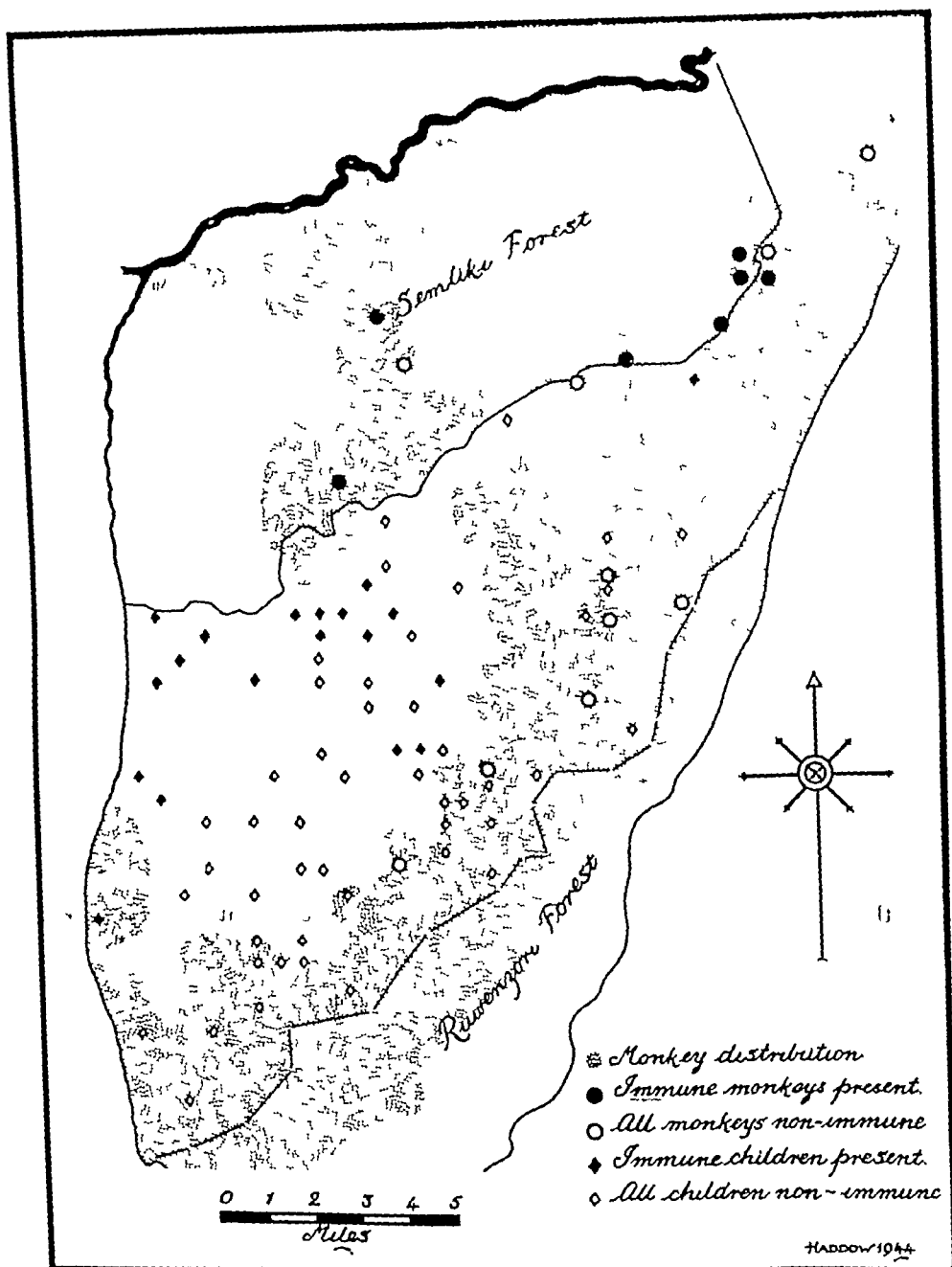


FIG 4—Sketch map of Bwamba County to show the distribution of immunity to yellow fever in monkeys ('minor species') other than the redtail and lowland colobus, and their known range, in relationship to the distribution of yellow fever immunity among children  
Details as in legend, FIG 2

## IMMUNITY IN RELATION TO SEX.

The crude data show a preponderance of immunity among females, but when the differences in age composition of the sex samples are eliminated, the frequencies of Table IV result. From these it is clear that there is no difference between the males and females, 61 per cent. of the former and 64 per cent. of the latter being protection-test positive. The  $\chi^2$  test shows that there is a probability of 0.73 that the existing small differences could arise from chance.

## IMMUNITY IN RELATION TO SPECIES.

Only two species have samples adequate in numbers for comparison consequently the tabulation (Table V) has been made in three categories, all the small samples having been combined under the heading minor species.

TABLE V  
IMMUNITY AMONG BWAMB MONKEYS WITH RESPECT  
TO SPECIES, CORRECTED TO STANDARD POPULATION.

Species.	Immune	Non-immune.	Total.
Redtail	23	16	49
Lowland colobus	29	27	66
Minor species	20	18	38
Totals	92	53	145

$$\chi^2 = 1.185 \quad - 2 \quad P = 0.57$$

Reference to Table I (page 688) shows that this group is heavily weighted by the baboons. After variations due to difference in age composition of the species samples have been eliminated, it is evident that there are no significant differences among the three groups listed.

The information available is not sufficient to permit a generalization concerning all species. Some of those which might well show marked differences in rate of immunity (such as the grey monkey and l'Hoeft's monkey) are represented by very few individuals. It may be concluded, however that among species important in relation to yellow fever in Bwamba any differences which may exist are negligible.

## IMMUNITY IN RELATION TO AGE.

In considering the incidence of immunity to yellow fever in relation to age, we have been handicapped by the scarcity of data concerning the duration

of the successive age periods in African monkeys. The best guide available to us at the moment is to be found in the data given by GILMORE (1943) for the South American tufted cebus (*Cebus fatuellus*). GILMORE's age grades are as follows

*Infant 1*—Incomplete deciduous dentition, 1 to 6 or 8 months

*Infant 2*—Complete deciduous dentition, 6 or 8 to 14 or 18 months

*Juvenile*—Mixed deciduous and permanent dentition, 14 or 18 to 36 or 40 months

*Sub-adult*—Incomplete permanent dentition, 36 to 42 months

*Adult*—Unworn complete permanent dentition, 36 or 42 to 96 or 120 months

*Old Adult*—Worn permanent dentition, 96 or 120 months to 180 or 240 months

For field work we have used a simplified scheme based on GILMORE's subdivisions, recognizing the following age grades

*Juvenile*—Incomplete or complete deciduous dentition, 0 to 15 years

*Sub-adult*—Mixed deciduous and permanent dentition or incomplete unworn permanent dentition, 15 to 30 years

*Adult*—Unworn complete permanent dentition, 30 to 100 years

*Old*—Worn permanent dentition, 100 to 200 years

In Table I the results of the whole survey are given in detail, with regard to species and age grade, and in Table VI the main results are summarized

TABLE VI  
IMMUNITY AMONG BWAMBA MONKEYS WITH RESPECT TO AGE GRADES

Age grade	Class midpoint years	Immune	Non-immune	Total	Per cent immune
Juvenile	0 75	1	24	25	4
Subadult	2 25	7	16	23	30
Adult	6 50	63	18	81	78
Old	15 00	21	0	21	100
Totals		92	58	150	61

The analysis of these data having shown that the variation between species is insignificant, all data have been combined to permit study of the distribution of protective sera with respect to estimated age. The sample thus becomes large enough for the relative frequencies to be stable quantities.

When the relative frequencies of positive sera are plotted at the mid-points of each age grade, the distribution of Fig 5 (p 696) is obtained, the form of which at once suggests the possibility of there being a more or less constant rate of exposure. This hypothesis may be tested under the following considerations



Let  $y$  = proportion of protection-test positives  $t$  = time in years  $k$  = rate of increase of positives with time.

Then, by the hypothesis advanced

$$\frac{dy}{dt} = k(1 - y)$$

From which, by integration,

$y = 1 - ae^{-kt}$  where  $a$  is derived from the constant of integration.

When the function passes through the origin,  $a = 1$  and the curve is the usual catalytic one with the assumption that all of the normal monkeys are susceptible to yellow fever. The fitting of this curve and its interpretation have been discussed for human data by MUENCH (1934), who presents also a simple technique for fitting. Where the function does not pass through the origin, then  $a$  will have a value other than unity

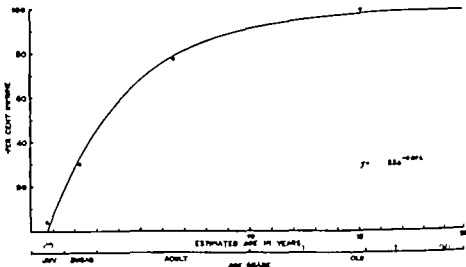


FIG. 5.—The incidence of immunity to yellow fever among Bwamba monkeys, with respect to age. The observed points are plotted at the mid point of each age grade. The theoretical curve is computed from the equation quoted above.

Using the values  $a = 1.23$  and  $k = 0.27$  an excellent fit is obtained, with the four observed points falling very close to the theoretical curve. The computed and observed data are given in Table VII. It is evident, therefore, that the hypothesis of a simple and constant exposure rate fits the Bwamba situation remarkably well especially when one considers the large class intervals into which the age judgments necessarily fall. This constant exposure may be either a truly continuous one or as seems more likely a frequently recurring series of episodes. However the actual spread of virus may occur it appears

that the susceptible monkey population of Bwamba becomes immunized at the rate of 27 per cent per year

A further point of interest lies in the fact that the theoretical curve becomes zero at 0.75 year rather than at the origin. This suggests that the infant monkey population is either not susceptible or not exposed to infection until near the end of the first year of life. There is abundant reason for thinking that the first alternative is the true explanation. Because of the high exposure rate at least 75 per cent of the baby monkeys of Bwamba are born of immune mothers. The observations of HOSKINS (1934) and SOPER *et al* (1938) have shown that in the rhesus monkey, as well as in man, offspring of immune

TABLE VII  
CALCULATED AND OBSERVED FREQUENCIES OF POSITIVE PROTECTION  
TESTS IN BWAMBA MONKEYS.  
 $y = 1 - 1.23e^{-0.27t}$

t Years	y Calculated	y Observed
0.75	0.00	0.04
1.00	0.06	0.30
2.25	0.33	
4.00	0.58	0.78
5.00	0.68	
6.50	0.79	
8.00	0.86	
10.00	0.92	1.00
12.00	0.95	
15.00	0.98	

mothers are passively immune (as shown by the mouse protection test) for about 6 months after birth. By the end of the first year they are all protection-test negative and presumably fully susceptible.

That this conclusion may also apply to Bwamba monkeys is shown not only by the data of Fig 5 but also by experience in the field. An infant colobus was brought to the field station by a native who had killed the mother. It could not have been more than 10 days old, as the umbilical stump was still unhealed, but it proved to be immune. This monkey has not been included among the 150 specimens discussed in this paper. On another occasion, a mother and infant colobus were brought down with one shot. The mother was immune to yellow fever but the infant, which was probably about 9 months old, gave a negative protection test. These observations suggest that in nature, as in the laboratory, congenitally acquired passive immunity does occur and that it persists for a few months only.

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THE BEHAVIOUR OF A SUDAN STRAIN  
OF *LEISHMANIA DONOVANI* IN *PHLEBOTOMUS PAPATASII*  
A COMPARISON OF STRAINS OF *LEISHMANIA*

BY

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A strain of *Leishmania donovani* was received from Dr R KIRK of the Stack Laboratories, Khartoum. It proved uniformly infective for Syrian hamsters which died from 3 to 4 months after inoculation.

Laboratory-bred sandflies, *P. papatasi*, were infected with this strain by feeding through a membrane on the flagellate stages in cultures on Locke-serum agar, or on leishmania forms in emulsions of heavily infected hamster spleen. The technique employed was that previously described by ADLER and THEODOR (1927).

INFECTION OF SANDFLIES FROM CULTURES

Suspensions of flagellates made up with saline and inactivated rabbit blood and varying from 200 to 2,000 active flagellates per 0.1 c mm were used. The sandflies were re-fed on human beings or on normal hamsters. In the case of experiments with emulsions of infected spleen accurate counts were not made but it was roughly estimated that they contained about 500,000 parasites per 0.1 c mm. Sandflies fed on these emulsions were re-fed on

normal hamsters. In some experiments the emulsions were made up with 2 per cent. saline and kept at 30 °C. in order to determine whether this procedure had any influence on the behaviour of the parasites in the sandfly (In the case of *L. tropica* transmission by bite of *P. papatasi* was readily achieved by this treatment. (ADLER and BER, 1941).)

The behaviour of the flagellates in the sandfly is summed up in the following Table I.

TABLE I.  
SHOWING BEHAVIOUR OF SUDAN STRAIN OF *L. donofayi* IN *P. papatasi*.

	Number of flagellates per 0.1 c.mm.	Time of dissection in day after infecting feed and number of sandflies positive.							
		2	3	4	5	6	7	8	9
1	200 to 300	—	—	8 out of 14	—	—	—	—	—
2	400 to 500	1 out of 1	4 out of 5	4 out of 8	8 out of 21	8 out of 16	1 out of 5	6 out of 6	4 out of 29
3	800	—	—	—	5 out of 10	1 out of 1	4 out of 11	—	—
4	1 000	—	1 out of 1	—	—	3 out of 4	7 out of 8	2 out of 4	2 out of 1

*Remarks on Table I*

(1) In four sandflies the flagellates were confined to the stomach. In one sandfly with heavy infection they were found in the lower part of the cardia.

(2) In five sandflies with heavy infections flagellates were found attached to the oesophageal valve, in one after 2 days, one after 5 days, in two after 6 days and one after 9 days. In four sandflies the infection extended to the lower part of the cardia, in one after 3 days, in one after 4 days, in one after 5 days and in one after 6 days. In the remaining twenty-one positive sandflies the infection was confined to the stomach.

(3) In two sandflies, both after 5 days, flagellates were found attached to the oesophageal valve. In one after 6 days the lower part of the cardia was invaded. In the remaining seven positive sandflies the infection was confined to the stomach.

(4) In two sandflies, both after 7 days, flagellates were found attached to the oesophageal valve. In three sandflies, one after 6 days and in two after 7 days, flagellates reached the middle of the cardia. In the remaining ten positive sandflies the infection was confined to the stomach.

There were no cases in which the infection was confined to the cardia and in every case of infection in the cardia the stomach was heavily infected. In eight of the sandflies in which the infection was confined to the stomach flagellates were very numerous and multiplication had obviously taken place. It is therefore evident that failure to ascend the cardia does not depend only on the number of flagellates in the stomach.

## INFECTION OF SANDFLIES FROM EMULSIONS OF SPLEEN

A total of 140 sandflies were fed on suspensions of leishmania from spleens of heavily infected hamsters, of these fifty-three were fed on suspensions made up with normal saline and defibrinated rabbit blood and eighty-seven on inactivated rabbit blood. The former group of sandflies was kept at a temperature of 26° C and the latter at 30° C. No difference in the infection rate or distribution of flagellates was noted between the two groups. Refeeding on hamsters also had no effect on the infection and the details of the refeeds are therefore not recorded. The results of the dissections carried out at intervals of 1 day are as follows —

After 1 day Thirty-seven sandflies dissected, all positive. In thirty clumps of leishmania and non-motile flagellates with a flagellum of about 1 to 2  $\mu$  protruding from the extremity were found. In seven very active flagellates were found and in one they had invaded the lower part of the cardia.

After 2 days Twenty-two sandflies dissected, all were heavily infected and the whole cardia was choked with flagellates.

After 3 days Thirty sandflies dissected, in twenty-nine the stomach and cardia were choked with flagellates, in one the stomach was heavily infected and flagellates had invaded the lower part of the cardia.

After 4 days Nine sandflies dissected, in eight the stomach and cardia were choked with flagellates, one sandfly was negative.

After 5 days Eight sandflies dissected, all were heavily infected, the stomach and cardia being choked with flagellates.

After 6 days One sandfly dissected and found heavily infected, the stomach and cardia were choked with flagellates.

After 7 days Thirteen sandflies dissected, in four the infection was confined to the stomach and in seven the cardia was also choked and two were negative.

After 8 days Four sandflies dissected, two were found heavily infected, the stomach and cardia choked with flagellates, two were negative.

After 9 days Six sandflies dissected, in three the stomach and cardia were choked with flagellates, in two the infection was confined to the stomach, one was negative.

After 10 days Four sandflies dissected, the stomach and cardia were found choked with flagellates in three, one was negative.

After 11 days Two sandflies dissected, in one the stomach and cardia were choked with flagellates, one was negative.

After 12 days One sandfly dissected, negative.

After 15 days One sandfly dissected the stomach and cardia were found choked with flagellates.

After 16 days Two sandflies dissected negative.

## MORPHOLOGY OF FLAGELLATES IN SANDFLY

There is nothing particular to report on the morphology of the flagellates in the sandfly.

After 24 hours the ingested leishmania enlarge up to  $6\mu$  by  $3.5\mu$ . The development of the flagellum can be followed at first it is a non-contractile rod protruding from the anterior end and becomes contractile when its length approaches  $3.5\mu$ . Up to this point the flagellates are motionless. They do not develop simultaneously after 24 hours, in addition to enlarged leishmania forms and motionless flagellates with a very short non-contractile flagellum, sluggish forms with a body  $5.7\mu$  by  $3.5\mu$  and a flagellum  $3.5\mu$  to  $5\mu$ , and a few very active forms 8 to 9 long with a flagellum up to  $7.5\mu$  are found.

After 48 hours leishmania forms are either scanty or absent and very active flagellate forms from  $9\mu$  to  $23\mu$  (length of body without flagellum) are found. Forms, with body length  $16\mu$  to  $20\mu$  are very numerous. After 3 days the shortest flagellates are  $11\mu$  to  $13\mu$ , many are  $17\mu$  to  $24\mu$ , and a few reach dimensions of  $27\mu$  to  $36\mu$  in body length—i.e., much larger than any forms found in culture on Locke-serum-agar. Subsequently there is no change in the type of flagellates found. Short thin forms  $4.7\mu$  to  $10\mu$  in length with a flagellum longer than the body which are occasionally found predominant in the case of *L. infantum* in *P. perniciosus* and *L. tropica* in *P. sergenti* were not found. Since these forms invade the proboscis more frequently than others in the case of *L. infantum* they may be expected to occur in the vector of kala-azar in the Sudan (probably *P. langeroni*).

## LOCALIZATION OF FLAGELLATES IN CHITINOUS PARTS.

In serial sections of sandflies fed on leishmania, flagellates were found in the buccal cavity 3 days after the infecting feed. Only two instances of proboscis infections were found, one 4 and the other 5 days after the infecting feed.

## FEEDING EXPERIMENTS ON INFECTED HAMSTERS.

Twenty laboratory-bred sandflies were fed on a heavily infected hamster. All were eventually found negative. Thirty-one were fed on another heavily infected hamster two were found infected, one 3 days and the other 4 days after the infecting feed. In both cases the infection was confined to the stomach.

## INFECTIVITY OF FLAGELLATES FROM SANDFLIES.

22.6.44 Fourteen sandflies fed on a suspension of leishmania in normal saline and inactivated defibrinated blood.

25.6.44 Four sandflies were dissected and found heavily infected the cardia was choked with flagellates.

S ADLER

The midguts of all four sandflies were dissected out and introduced with dissecting needles into a point on the skin of the abdomen of two hamsters 5145. The animals were laparotomized and spleen smears showed a very heavy infection of leishmania in both cases.

Between 27.6.44 and 24.7.44 a total of forty-nine infected sandflies (six from cultures and forty-three from leishmania) were fed on four hamsters, 125 refed were distributed among the above sandflies of which 103 were from 3 to 12 days after the infecting feed, the hamsters were examined on 5.1.45—spleen smears and cultures were negative.

### Discussion

On purely epidemiological grounds *P. papatasi* can be excluded as a carrier of kala-azar in the Sudan. It is also evident that this sandfly feeding on heavily infected and moribund hamsters either does not become infected at all (in the majority of cases) or develops a slight infection which does not extend significantly to the anterior position. Nevertheless, the above experiments are of interest in so far as they permit an analysis of the factors involved in the host-parasite relationship between various species of sandflies and the strains of *Leishmania* which they transmit, in contrast to those which they do not transmit but with which they can be infected in the laboratory by the administration of such massive doses of parasites as are hardly likely to be ingested under natural conditions.

Whereas all known artificial media which are suitable for one strain of *Leishmania* are equally suitable for any other strain, the midgut of different species of sandflies shows a specific selectivity for special strains. This specificity is manifested in two ways—the number of parasites necessary to establish an infection and the behaviour of the flagellates once the infection has been established. In the case of *L. infantum* in Malta, an infection rate of 30 per cent may be produced in *P. perniciosus* by feeding on a dog with skin infection so slight as to be hardly detected by histological examination, and in *P. major* (Sicilian strain) feeding on the same animal the infection rate is 80 per cent (Thirty-one out of 119 sandflies in the first case and twenty-five out of thirty-one in the second), whereas *P. papatasi* feeding on the same animal does not become infected at all (ADLER and THEODOR, 1935). The infection once established in *P. major* or *P. perniciosus* is maintained throughout the whole life of the sandfly in the laboratory and there is no fall in infection rate with time. The flagellates progress to the anterior position in a large percentage of sandflies (70 per cent in the case of the Maltese strain of *L. infantum* in *P. perniciosus* and approximately 100 per cent in the case of the Catania strain). The behaviour in the sandfly is not determined only by the intensity of the infection for even in very slight infections flagellates may reach the oesophageal valve.



In the case of *L. tropica* strains from different regions differ in the infection rate produced in *P. papatasi* as can be seen from Table II.

TABLE II.

Origin of <i>L. tropica</i> strain.	Number of flagellates per 0.1 c.mm.	Number of sandflies fed	Number infected.
Jericho	100	70	16
	300	80	53
	400	45	44
Baghdad	100	19	8
	300	92	38
	7-800	34	18

The above differences in infectivity for *P. papatasi* are apparently inherent in the strains of *Leishmania* and do not depend on the host. It was shown (ADLER, 1937) that two strains isolated at an interval of 2 weeks from a human being infected experimentally with a Baghdad strain (for the purpose of vaccination against oriental sore prior to taking up residence in Jericho) and subsequently infected naturally in Jericho (while the lesion from the experimental inoculation was still active) could be distinguished by the differences in infection rates in sandflies fed on suspensions of flagellates.

The infectivity of Cretan strains of *L. tropica* for *P. papatasi* fed on human lesions was very slight as compared to that for *P. sergenti* or even *P. major* (four out of forty-five in *P. papatasi* fifty-two out of seventy-seven in *P. sergenti* and fourteen out of fifty-five in *P. major*) and was even lower than that of the Baghdad strain for *P. papatasi* (ADLER, THEODOR and WITENBERG 1938).

In spite of the differences in infectivity of various strains of *L. tropica* for *P. papatasi* there is uniformity in the forward trend of the flagellates even in slight infections.

The factors responsible for these differences in the infectivity of various strains for *P. papatasi* are not known but in the case of the Cretan strain there is an indication that it is influenced by the amount of serum ingested during a meal as the figures in Table III indicate (ADLER 1938).

In the case of the Palestinian strains varying the amount of serum in the suspension has no influence on the infection rate.

It is instructive to contrast these findings with those obtained with the Sudan strain of *L. donovani*. Examining the results of dissections of infected sandflies on cultures it appears impossible to ascribe a definite infection rate for sandflies feeding on suspensions containing from 200 to 1,000 flagellates per 0.1 c.mm. because the infection rate diminishes with time. There is a definite tendency for the infection to disappear and since every individual sandfly

obviously ingested flagellates (which do not descend to the hindgut and rectum unless the infection is very heavy) it is obvious that they are destroyed in the stomach. It is, therefore, justifiable to speak of an active natural immunity in the sandfly *P. papatasi* against this particular strain of leishmania.

In a considerable number of sandflies infected on culture (forty-two out of sixty) the infection was confined to the stomach. This failure to ascend towards the oesophageal valve was independent of time, for purely stomach infections were found at all intervals between 2 and 9 days after the infecting feed, and within wide limits was also independent of intensity of infection since pure stomach infections were found in cases where there had been obvious multiplication of flagellates. In the sandflies infected on suspensions of leishmania containing somewhere about 500,000 parasites per 0.1 c mm (i.e.,

TABLE III  
SANDFLIES (*P. papatasi*) FED THROUGH MEMBRANE ON CRETAN STRAIN OF *L. tropica*

Number of flagellates per 0.1 c mm in suspension in 10 per cent rabbit serum	Number of sandflies fed	Number positive	Number of flagellates per 0.1 c mm in suspension in 50 per cent rabbit serum	Number of sandflies fed	Number positive
100	19	8	—	—	—
300	7	7	—	7	1
—	—	—	350	13	4
—	—	—	1,100	51	6
1,600	16	15	1,600	11	3
—	—	—	1,900	—	—

in rather less than half the volume of an average feed of *P. papatasi* with an empty alimentary tract) the results were quite different. In insects dissected after 24 hours the majority of the parasites still in the leishmania stage were found in clumps scattered throughout the contents of the stomach. After 48 hours the stomach was a seething mass of active parasites and the whole of the cardia was packed with flagellates. Obviously the flagellates had multiplied till they had taken up most of the available space in the midgut. In some of these intense infections active parasites had also descended to the hindgut and the rectum. After 3 days the infection had progressed to the buccal cavity. Only a relatively small number of sandflies (ten out of 140) freed themselves of the infection and in a few (six out of 103 dissected after 2 to 6 days) the flagellates were confined to the stomach.

It is quite clear that the immunity of the sandfly had been broken down by a massive dose of parasites which had not only multiplied rapidly but, in contrast to many sandflies infected by the smaller doses of flagellates, had produced a persistent infection which in the large majority of cases had

quickly progressed anteriorly. The factors which tend to confine the infection to the stomach had also been eliminated and the behaviour of the flagellates in *P. papatasi* had approximated to that expected in a transmitting sandfly (in the case of the Sudan strain probably *P. langeroni*).

The behaviour of the Sudan strain in *P. papatasi* is rather similar to that of Italian strains of *L. infantum* and Indian strains of *L. donovani* both of which produce a relatively low infection rate in *P. papatasi* when fed on suspensions containing the same number of flagellates per 0.1 c.mm. which is sufficient in the case of Palestinian strains of *L. tropica* (Adler and Theodor, 1931) to produce an infection rate of approximately 100 per cent. However there appears to be a difference between the Indian and Italian strains in that the latter though producing a low infection rate in *P. papatasi* will when once established, progress anteriorly to the oesophageal valve while the former in two Indian strains examined is mainly confined to the stomach (Adler and Theodor, 1931). A strain from a case of South American visceral leishmaniasis resembled *L. infantum* in its behaviour in *P. papatasi* rather than *L. donovani* (Adler and Theodor, 1938).

#### BEHAVIOUR OF FLAGELLATES IN *P. papatasi* AS AN AID IN DISTINGUISHING STRAINS OF *Leishmania*.

During the last 20 years we have had the opportunity of examining numerous strains of *L. infantum*, *L. tropica* and *L. donovani* the majority isolated by ourselves and others received from various laboratories. In dealing with a large number of strains we were naturally faced with the problem of how far they could be distinguished by morphology, cultured method, animal inoculation and behaviour in sandflies. It is well known that in smears from human material it is quite impossible to detect any morphological differences between *L. donovani*, *L. infantum* and *L. tropica*. WENTON (1926) states,

Little assistance has been obtained from animal inoculations for it has been found that *L. donovani* which produces a general infection in man may give rise to purely cutaneous lesions in animals as also in man while *L. tropica* which causes local cutaneous lesions in man may produce generalized infection in animals. HOWE (1943) includes all the human strains within a single species, *L. donovani*. Howe's position is briefly as follows: he admits the obvious biological differences among human strains causing diverse clinical conditions but maintains that, since these biological characters are of no taxonomic value they cannot be used for designation of species. He therefore proposes an independent taxonomic status for biological races of parasitic protozoa. If this argument is followed to its logical conclusion the leishmanias of lizards *L. hemidactyli* and *L. agassiae* should also be included in *L. donovani*.

It seems to us essential to distinguish the different types of human and animal leishmanias by some system of nomenclature because the differences between them are real, constant and of the greatest importance. Whereas the





FIG. 1 *Leishmania donovani*  
Not uniform character of  
growth.



FIG. 2 *Leishmania tropica*.  
Note granular character of  
growth.

DROP OF CULTURE ON LOCKIE SERUM A 45

organism causing cutaneous leishmaniasis produces a relatively harmless cosmetic defect not affecting the general health, the one causing visceral leishmaniasis kills the overwhelming majority of untreated cases. The causative agent of oriental sore *never* produces a serious visceral infection in man. KATZENELLENBOGEN (1942) inoculated eighty-two human beings from macerated spleens of Syrian hamsters heavily infected with *L. tropica* and cutaneous lesions only were produced.

We think that the different leishmanias *can* be distinguished by animal inoculation. *L. tropica* in Syrian hamsters uniformly produces a visceral infection\* and a uniform skin infection (without ulceration) as intense or even more intense than that produced by *L. donovani* or *L. infantum*, but in the case of *L. tropica* the parasites in the hamster are much larger than *L. infantum* or *L. donovani*, and there is no difficulty in distinguishing spleen smears of the two types of infection. (In *L. tropica* in the Syrian hamster organisms  $5.6\mu$  by  $3\mu$  are quite common, whereas in *L. infantum* and *L. donovani* they seldom attain  $4.2\mu$  by  $2.8\mu$ .)

All the strains (more than 200) we have personally isolated from cases of cutaneous leishmaniasis could be distinguished from those we have isolated during the last 20 years from cases of human and canine visceral leishmaniasis in Palestine, Malta, Italy, Greece and N. Africa (about sixty strains) and from strains sent to us from India, S. America and China. In all strains flagellates grow in a layer near the surface on Locke-serum-agar. A small loopful of culture placed on a slide (without a coverslip) and examined with a low power is seen to consist of thick granular masses of flagellates separated by less dense growth in the case of cutaneous strains, while in the visceral strains the flagellates are almost uniformly diffused throughout the drop (Plate, Figs 1 and 2). (In *L. donovani* from India the growth is rather thicker than in *L. infantum*.) However, one strain of *L. infantum* sent by the late Professor MESNIL in 1927 originating from Tunis consistently grows like the cutaneous strains. (We still maintain this strain in culture.)

If we take an arbitrary standard of 300 flagellates per 0.1 c. mm., the visceral strains produce an infection rate not approaching 50 per cent (and usually less than 25 per cent) in *P. papatasi*. The above mentioned strain of *L. infantum* from Tunis which resembles *L. tropica* in cultural characters, infected twenty seven out of thirty-four *P. papatasi* fed on suspensions of 200 to 300 flagellates per 0.1 c. mm.

We have had under observation, since 1928, a canine strain of visceral leishmaniasis from the Pasteur Institute, Tunis. This strain also resembles *L. tropica* in its cultural characters and also produces a relatively high infection rate in *P. papatasi* (twenty two out of fifty nine in suspensions not exceeding 100

\* FULLER and GERMAN (1942) state that *L. brasiliensis* produces local lesions only in the Syrian hamster. This constitutes an interesting biological difference between *L. tropica* and *L. brasiliensis*.

flagellates per 0.1 c.mm.). In referring to this canine strain ADLER and THOMPSON (1931) noted that it was particularly infective for mice in which it produces local lesions after inoculation into the tail. We also succeeded in producing local lesions in a human being by inoculating material from the tail of an infected mouse. It should be stressed that in the light of all subsequent experience the production of local lesions in the tails of mice is characteristic of strains of *L. tropica*.

We were puzzled by these findings which prevented us from concluding years ago that *L. infantum* and *L. tropica* are distinguishable by their cultural characters. Apart from these two strains the *L. infantum* strains present a uniform picture in cultures on Locke-serum-agar. We have at present (in addition to the two abnormal strains) the following strains of *L. infantum* under cultivation: six canine strains (three from Palestine, two from Greece and one from Malta) and twenty-one human strains (two from Greece, one from Cyprus, four from Malta, four from Palestine, one from N. Africa, six from Italy, two from Sudan and one from Uzbekistan). All of them can readily be distinguished in culture from twenty-one strains of *L. tropica*. Three strains from cases of visceral leishmaniasis from Brazil and one from China are indistinguishable from *L. infantum* in culture.

MAYER and RAY (1928), working with five strains, also found differences in cultural characters on Nöller's medium.

It is interesting to note that other abnormal strains also originating from North Africa have been recorded by YOUNG and HARMON (1927). These authors worked with five strains from Tunis where they had been under cultivation for periods varying up to 15 years and had originally produced visceral lesions only. Two strains were of human origin, two from visceral infections in dogs and one from a gecko *Tarentola mauritanica*.

They found that inoculation of these strains into Chinese hamsters produced a visceral infection but, after a lapse of from 2 months to over a year, swellings of the joints, testes, clitoris, infiltration and ulceration at the base of the tail, nose and margins of the ears occurred. With the appearance of the external lesions the visceral infection diminished. HINDLE and THOMPSON (1928) made similar observations with a North African human strain of *L. infantum*. During the last 15 years we have examined many strains of *L. infantum* both human and animal from Sicily, Malta, Greece and Palestine and found that both in Chinese and Syrian hamsters a persistent visceral infection is produced.

We cannot account for the above discrepancies though several possibilities suggest themselves: either the abnormal strains were originally *L. tropica* or if they were *L. infantum* they had mutated and become indistinguishable from *L. tropica*. The infection of hamsters with a strain of leishmania from *Tarentola mauritanica* is even more difficult to understand. ADLER and THOMPSON (1930) failed to infect geckoes by inoculating infected bone-marrow from a case of infantile kala-azar and from cultures of *L. infantum*. The same authors

(1929) had previously reported on the behaviour of two strains of *L. tarentola* received, one from Tunis and one from the Pasteur Institute, Paris. Both strains adopted an anterior position in *P. papatasi*. We still have these strains and they resemble *L. tropica* in their cultural characters.

The study of a large number of strains isolated personally, justifies the conclusion that the cutaneous and visceral leishmanias occurring naturally in man and dog in the Mediterranean basin differ in their cultural characters, pathogenicity in their natural hosts, behaviour in animals and in sandflies. Indian visceral strains can also be readily distinguished from cutaneous ones by behaviour in Syrian hamsters and sandflies.

The visceral strains differ among themselves in susceptibility to organic antimony compounds and to aromatic diamidines in both hamsters and human beings. Infections caused by the parasite of Indian kala azar are more easily eradicated than those due to the parasite of Mediterranean visceral leishmaniasis.

#### SUMMARY AND CONCLUSIONS

Sandflies, *P. papatasi*, were infected with a strain of *L. donovani* from the Sudan by feeding on suspensions of flagellates and on leishmania from the spleens of infected Syrian hamsters. In sandflies infected on cultures (200 to 1,000 flagellates per 0.1 c mm) the infection tends to die out in a few days. In sandflies infected with large numbers of leishmania (ca. 500,000 per 0.1 c mm) permanent infections were established and the flagellates reached the buccal cavity after 3 days.

Flagellates from *P. papatasi* infected with *L. donovani* (Sudan strain) produced a generalized infection in two Syrian hamsters after intracutaneous inoculation.

Heavily infected sandflies, *P. papatasi*, fed on normal hamsters gave negative results.

The behaviour of various strains of leishmania in *P. papatasi* is discussed.

*L. infantum* can readily be distinguished from *L. tropica* by cultural characters, by the relatively large size of the leishmania forms of *L. tropica* as compared with those of *L. infantum* (and *L. donovani*) in the tissue of the Syrian hamster, and by behaviour in sandflies.

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## MUCOCUTANEOUS LEISHMANIASIS IN KENYA

(WITH A NOTE ON PENICILLIN TREATMENT)

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Before the war, leishmaniasis was rare in East Africa. The Abyssinian campaign, however, resulted in outbreaks of kala-azar among African troops engaged in operations in Abyssinia (TOBIAS, 1941, ANDERSON, 1943, COLE, 1944), and in a number of cases cutaneous lesions, mostly of the post-kala-azar leishmanoid type, were observed (COLE, 1942, 1944). One of COLE's cases, an African soldier who had been in Abyssinia for a year, presented a purely cutaneous type of infection, somewhat resembling oriental sore. Similar cutaneous cases were seen in the first war years by MANSON-BAHR (1945) in Italian internees from Abyssinia, in Indian soldiers recently transferred to Kenya, and by the writer in 1943 in a Rumanian refugee who had lived in Palestine before he travelled to East Africa. All these cases were obviously "imported" ones, the inference was that the infections were contracted in endemic areas outside Kenya and, owing to the long period of incubation for dermal leishmaniasis, only became manifest in East Africa.

The only area in which leishmaniasis was known to be endemic before the war was the Northern Frontier District, situated between the Uaso Nyiro

\* The writer's thanks are due to the Acting Director of Medical Services, Kenya Colony, for permission to publish this report, to Dr G L TIMMS, pathologist, Medical Research Laboratory, and to Dr W G KERR and Mr ARTHUR MBUTHIA, DIP MED B A, of the Native Civil Hospital, Nairobi, for their kind assistance.

river and the Abyssinian border a few cases of kala azar were reported from there prior to 1940. (ANDERSON 1943) It may therefore prove of interest to record an apparently autochthonous case of mucocutaneous leishmania infection from a district in Central Kenya in which a number of instances of visceral leishmaniasis have occurred quite recently.

### CASE HISTORY

The patient was male Mkenba, aged about 35 from Kisa, location between Machakos and Kitui, the two main centres of the Ukamba reserve east of Nairobi. The patient lived there until he went to Kiambu (near Nairobi) to work on a farm, after few months he returned to Kisa which he left again for Nairobi in January 1945. He took up work with the railway and lived at the railway landies near the town. The present condition began to develop shortly after his arrival in Nairobi and he was admitted to the Native Civil Hospital on 12th September 1945.

On admission he was found to be in rather poor state of nutrition. The temperature and pulse were normal. Examination of the inner organs revealed nothing of interest. The spleen and liver were not enlarged.

The face of the patient was greatly disfigured by diffuse swelling of the upper lip and of the cartilaginous parts of the nose. The skin of these parts was deeply infiltrated; the epidermis was thinned and scaly showing some superficial erosions, mainly in the region of the philtrum, from which sero-purulent fluid exuded. The hair of the upper lip had disappeared from the affected parts. The nostrils were much distended, the right nostril was blocked and expanded by polypus-like mass which protruded from it, bled easily on touch, and made the insertion of a speculum impossible (Fig. 1). The left nostril was more accessible; the mucosa was swollen, partly eroded and covered by thin easily removable crusts. The mucous membranes of the mouth and pharynx were not affected; the lymphatic glands of the neck appeared to be slightly enlarged but not tender. The whole condition was apparently quite painless.

### Laboratory Findings

Kahn reaction	negative.	Polymuclears	64 per cent.
Blood count	R.B.C. 4,280 000	Lymphocytes	32
	W.B.C. 7 000	Monocytes	2
	C.I. 1.05	Eosinophils	2
	N. malaria parasites present.		

Sternal puncture N. abnormalities of cell picture no parasites.

Sub-epidermal puncture of the infiltrated skin polymuclear and macrophage cells. The protoplasm of the latter contains large numbers of leishmania of varying size and shape. Occasionally they are found extra-cellularly. Smear from erosion of the upper lip polymuclear cells and cocci.

### Histopathology

(Biopsy from the right side of the upper lip.)

The epidermis is covered by loose parakeratotic masses, the spaces between which are filled by fibrin and contain many polymuclear cells.

The rete Malpighii shows marked intercellular (spongiosis) and intra cellular oedema, and is in most places reduced to five to six layers of cells. The rete pegs are flattened out, but in some places irregular epithelial proliferation into the corium is notable (acanthosis). Polymuclear leucocytes are frequently encountered in the intercellular spaces. In one instance a large



used in syphilology but a division into primary secondary and tertiary lesions appears to be successful only in the case of dermal lesions associated with kala azar. Of the various purely cutaneous and ulcerous mucocutaneous forms not enough is known as yet to permit systematizing. The differentiation of the clinical types is far from accomplished and there are too many instances of transitional and borderline types.

In the case presented here, certain features are reminiscent of American (ulcerous, mucocutaneous) leishmaniasis but the resemblance is merely superficial. The involvement of the nasal mucosa may be either primary or due to a continuous spread from the infected skin of the upper lip. The nasal lesions are certainly not as destructive as those of American leishmaniasis the pharynx is not affected and the lesions seemed to respond to treatment much more readily than typical American leishmaniasis and some of the severe ulcerations seen in the Sudan (HUGHES and MAYNE, 1935). It may be noted that nasal polypus has been described as a rare complication of the American form by COSTA (1944) and other Brazilian workers.

The cutaneous infiltration of the upper lip was of a type not infrequently seen in tuberculosis of the skin—typical forms of oriental sore resembling lupus tumidus have indeed been described by Italian workers (MONACELLI 1936 NICOLAU and MASSIA, 1930). The histological picture, however showed no signs of a tuberculoid structure—which again is unusual considering the long duration (8 months) of the infection. Other unusual microscopical features were the presence of a large macrophage cell, loaded with parasites, in the middle of the epidermis while cells of this type have occasionally been found lying between cells of the basal layer (MC CARTHY 1931) they seem to be generally unwilling to travel further through the uncongenial territory of the rete Malpighii—unlike the polynuclear cells. Endothelial proliferation in the deeper skin vessels is another uncommon finding.

Epidemiologically the interest of this case lies in the facts that the patient came from an area which until recent times was thought to be free from all types of leishmanus infection, and that it is the first case of cutaneous leishmaniasis in Africa south of the equator. WRIGHT (1943) when presenting a case of kala azar in a native of Kenya who had contracted the infection in Abyssinia, referred to two more kala-azar patients whom he had recently treated and who never had left their homes in the Kamba reserve near Kitui. Since then six more cases of kala-azar have been treated in the Native Civil Hospital, Nairobi. Five of these patients were Wakamba from locations near Kitui who had not been outside the reserve. The Kamba reserve is a densely populated and easily accessible district south of the equator and east of the Nairobi Nanyuki railway line. It does not border directly upon the Northern Frontier District north of

Fox (1931) recommends replacing the term *espundia* which is local name used in Peru only but unknown in other American countries by the more comprehensive "American leishmaniasis."

the equator which<sup>1</sup> was known as an endemic district before the war. The location from which the present case came is nearer to Machakos than to Kitui, and so far no cases of kala-azar have been recorded from this region. It cannot be decided at present whether these cases indicate the presence of a true endemic focus of long standing which became known only recently, or whether war conditions (movements of, ? infected, askaris on leave, training areas near the reserve, etc.) may have caused a spread of the infection from old endemic foci to the Kamba reserve. There were many Wakamba among the askaris of the East African army, and the neighbourhood of the reserve was the place of considerable military activity during the Abyssinian campaign and afterwards. It may be noted in this connection that in the Sudan, a country in which leishmaniasis has been studied intensely for decades by experienced workers, a new endemic area was discovered more recently (STEPHENSON, 1940). The occurrence of dermal leishmaniasis in an area near a focus of kala-azar parallels the regional coincidence of dermal and visceral leishmaniasis seen in the Sudan by KIRK (1942-44). The unusual character of the cutaneous lesions suggests the presence of an atypical strain of *Leishmania tropica*, or/and the possibility of secondary bacterial or fungous invasion of the lesions. This has been suggested in the case of American leishmaniasis by RUGE (1930).

The rationale of the penicillin treatment was not to attempt a *therapia magna sterilisans*—this has been tried before, without success (SNOW, 1944). The intention was rather to clear the way for an uninhibited action of antimony, as the possibility of a secondary infection was to be considered. The procedure was analogous to that suggested by HARGREAVES (1945, 1945a) who found that chronic cases of amoebiasis showed a better response to emetine after a preliminary treatment with penicillin and sulfa drugs. The results in the present case were not discouraging, and one may feel justified in exhibiting this treatment in similar instances of ulcerous mucocutaneous leishmaniasis.

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EXOERYTHROCYTIC SCHIZOGONY  
IN *PLASMODIUM KOCHI* LAVERAN  
A PRELIMINARY NOTE

BY

P C C GARNHAM

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Monkeys of the genus *Cercopithecus* are frequently found to harbour malarial parasites in the red blood cells, the parasites apparently being gametocytes of *Plasmodium kochi*. Some infections are heavy and consist not only of mature gametocytes but numerous young ring forms as well. It was thought that in such cases dividing forms must be found if a thorough search were made of the internal organs. Several complete postmortem examinations were performed, and smears and sections were made of the brain, spleen, liver, lung, heart, kidney, lymph glands, mesentery and bone marrow. Nothing of interest was seen until it was noticed that the surface of the livers was studded with white or translucent spots. The lesions varied in number from one to a 100 or more. As a rule the number was limited to about ten. The diameter of the largest spot or merocyst was 2 mm. The foci are most easily seen on the surface of the organ, but they exist also in the interior. Two kinds are recognizable, a white and a translucent, and these represent different stages in the development of the parasite. The translucent cysts are raised slightly above the surface of the liver. When incised a colourless fluid escapes and this liquid contains white flakes which have become detached from the internal surface. These flakes are just visible to the naked eye and are actually the developmental form of the parasite.

Smears made from the merocysts, stained with Romanowsky, present a characteristic appearance. Two types of structure are seen, red staining merozoites and schizonts of different sizes. The merozoites are usually in very large numbers, scattered widely throughout the smear. They are round or oval in shape, and appear to consist solely of chromatin. Owing to their softness they are liable to distortion and in smears are often drawn out into red strands of great length. The schizont varies in size from a small oval body about 4 $\mu$  long to large irregular masses 40 $\mu$  or more in length. In the younger or smaller forms, the cytoplasm is dense and of a deep blue colour and scattered



through its substance are the red staining nuclei or merozoites. As development proceeds the cytoplasm becomes finer more lightly coloured, and on maturation of the parasite disappears entirely leaving the nuclei bare. A highly characteristic feature of all but the smallest forms is the presence of one or more vacuoles within the schizont. There is no pigment and the parasites are apparently not inside any of the tissue cells of the host.

Serial sections of the liver foci indicate the more exact nature of the appearances seen in the smears. Three stages of development can be recognized which possibly may be classified as follows —

- (1) Cytomere formation.
- (2) Mature merocyst.
- (3) Various degrees of degeneration of the parasite, according to the local tissue reactions.

Macroscopically the translucent cysts correspond to (2) and the white foci either to (1) or (3).

The cytomere stage can be briefly described as the infolding of a plasmodial mass with a peripheral distribution of nuclei. The general picture bears a close resemblance to the development of *Haemoproteus* in the liver or kidney of the Baghdad sparrow (WERNOW 1926). This stage like the later ones, occurs in a localized sphere surrounded by liver parenchyma. Polymorphonuclear leucocytes are numerous around the cytomeres, and the subsequent course of events appears to depend upon the success or failure of these and other tissue cells to deal with the parasite. If the parasite wins, stage (2) ensues, by nuclear multiplication within the cytomeres, the disappearance of the thin walls of the latter and their eventual coalescence to form a cyst wall packed with merozoites. The cyst wall encloses a cavity filled with fluid in which strips of the coalesced cytomeres dangle and occasionally break free. The actual wall is a hyaline structure, perhaps derived from the cytoplasm of the cytomeres, which creeps for a short distance in fingerlike processes between the tissue cells.

These mature merocysts are surrounded by flattened liver cells and sometimes by a narrow border of fibroblasts but tissue reaction at this stage is as a rule negligible. The host reaction earlier is highly characteristic. The polymorphonuclear leucocyte response may be successful early on and all signs of the parasite disappear in what is in effect a small abscess. If the parasite manages to survive, the leucocytes retreat and the cyst becomes surrounded with a layer of fibroblasts and giant cells. Around the mature cyst little of this cellular reaction remains.

The beginning and end of the cycle are unknown, but it seems likely that each focus of cytomeres represents the product of the multiplication of a single sporozoite or merozoite whilst the merozoites of the mature cyst are in such close contact with the liver tissue that it seems probable that they escape via it into the circulation when they grow into gametocytes.

P C C GARNHAM

The reasons for thinking that the structures described above represent part of the cycle of *P. kochi* are as follow —

Animals exhibiting heavy blood infections were thoroughly searched for schizonts and the only forms to be discovered in smears were schizonts rather of the *gallinaceum* type, and in sections, cytomeres strongly resembling those of *Haemoproteus*

No other blood protozoa were found in these monkeys, except for one animal which developed a heavy *Babesia pitheci* infection following splenectomy

The merocysts bore little or no resemblance to any of the fungi and several cultures on Sabouraud were negative

Finally, seven monkeys whose blood had been repeatedly negative for malaria were sacrificed and the livers were discovered to be "spotless" Of sixteen positive monkeys, the livers of fifteen showed the characteristic picture, the single one with a negative liver had a very scanty infection of gametocytes in the blood stream

Experimental confirmation so far has not been obtained Cysts have been excised and ground in normal saline, and the suspension inoculated into the subcutaneous tissue and into the liver of clean monkeys with negative results Cysts have also been placed in defibrinated non-malarial monkey blood with a few drops of 50 per cent glucose and incubated at 37° C, but no infection occurred A slightly suggestive result was obtained by giving paludrine (0.2 gramme daily) to a monkey showing approximately one gametocyte per field of a thin film The day before starting the drug, the abdomen was opened and two spots were noted as being present on the liver (only part of the surface could of course be seen) The paludrine was continued for 4 days when the animal died At postmortem only one tiny spot could be found

#### DISCUSSION

The parasite under discussion is that generally known as *P. kochi* Laveran and bears a close resemblance to the figures of *kochi* gametocytes reproduced from the original plate by ABERLE (1945) The blood parasites also appear to be essentially the same as the *P. kochi* of the West Coast of Africa, slides of which I have recently been able to examine through the kindness of Dr F HAWKING In Kenya I have found the parasites in the following species of monkey —

<i>Cercopithecus aethiops centralis</i>	Neumann
" "	<i>johnstoni</i> Pocock
" "	<i>albotorquatus</i> Pousargues
" "	<i>kibonotensis</i> Lönnberg
" "	<i>kolbi</i> Neumann
" "	<i>nictitans schmidtii</i> Matschie

The type of schizogony described in this paper is more akin to that of *Haemoproteus* than *Plasmodium*. Even the exoerythrocytic schizogony of the latter is not very much like the *kochi* schizonts. It might be thought that *kochi* should be removed to the genus *Haemoproteus* but there are two reasons against this. Firstly *Haemoproteus* comprises parasites of non-mammalian nucleated blood with "Halteridium" gametocytes and, secondly whilst part of the schizogonic cycle resembles that of *Haemoproteus* (i.e., the formation of cytomeres) the later cystic stages are unique and quite unlike anything hitherto described in the Haemospondiidae.

Dr WENTON has drawn my attention to a paper by LEVADITI and SCHORN (1932) who described the appearance in section of these liver bodies in a baboon. They failed to associate them with malaria and called them *Hepatocystis summei*.

A full report on these parasites will be published later.

### SUMMARY

1 The livers of fifteen out of sixteen monkeys exhibiting *P. kochi* in the peripheral blood showed white or translucent spots which are thought to represent a stage in the development of the parasite. The livers of seven monkeys consistently blood negative were spotless.

2 Smears of these spots or merozoites contained numerous free merozoites and unpigmented schizonts of varying size. A characteristic feature of the schizont is the presence of one or more vacuoles.

3 Sections reveal the development of cytomeres which later coalesce to form a cyst wall packed with merozoites.

4 The host reaction is characteristic—at first, there is a great concentration of polymorpha, and later these are replaced by fibroblasts and giant cells.

I have great pleasure in thanking Dr C. M. WENTON and Dr CECIL HOARE for examining part of the material and for making various suggestions.

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## CORRESPONDENCE.

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### MALNUTRITION WITH OEDEMA IN THE GOLD COAST

*To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

I have recently read in the *Archives of Disease in Childhood*, June, 1946, an article by Dr B A S RUSSELL, of the Colonial Medical Service, Gold Coast, on the prevalence in Ashanti, Gold Coast, of a malnutritional illness affecting children in which the predominant signs are generalized oedema, which may be very slight or marked dryness of the skin and sparse hair on the scalp, ulcers on the legs or buttocks, and inflammation of the mouth. Diarrhoea is present or reported by the mother. The sufferers are young children, 2 to 4 years of age, and the mortality is high.

Dr RUSSELL refers *inter alia* to Dr C WILLIAMS' report on a similar illness near Accra (*Archives of Disease in Childhood*, 1933) and mentions the opinion generally held, that a protein deficiency is the major dietetic cause of the disease. A photograph of a fatal case which attended the Kumasi Welfare Clinic is included. Dr RUSSELL implies that the illness is common, but the number of cases seen in Kumasi is not stated, nor is it mentioned that any cases attending the Kumasi clinic resembled infantile pellagra such as other writers have described as a terminal complication of this condition.

My purpose in writing is to draw attention to the apparent lack of official cognizance of this well-known and grave disease which the appearance of Dr RUSSELL's article at the present time suggests. The condition was first described in the *Gold Coast Medical Report* in 1931 by Dr C WILLIAMS. In 1939 I published a monograph on *Diet and Ill Health in the Forest Country of the Gold Coast* (H K Lewis, Ltd), in which I recorded some 210 cases of childhood malnutrition, seen at Akim Oda Hospital, Ashanti, in 1935-36. Oedema was prominent in many of the sufferers, usually accompanied by diarrhoea. All the severe cases died, a high proportion of them developed a dark flaking dermatitis, typical of pellagra in distribution, before death. The composition of the local diet was studied critically. It showed the faults generally recognized, in particular, extremely low biological protein. Photographs of many typical cases were included.

In 1940-41 I carried out an official diet and nutrition survey of the Gold Coast. In my report I again drew attention to the prevalence of this pellagra type of malnutrition in Ashanti, and made certain recommendations, including a mutually beneficial exchange of red palm oil and ground nuts between Ashanti and the Northern Territories respectively (The N T diet is grossly deficient in Vitamin A). Ground nut cake (the nut residue from which the oil has been expressed) contains adequate amino-acids for the normal growth of the young

(However the report mentioned was officially suppressed, and this decided me to resign from the service.)

Concerning the pathology I believe that Dr WILLIAMS reported degenerative liver changes in some of the fatal cases in Accra. Permission for post mortem examinations in Oda was not obtained. However it is interesting to recall CLARKE's experiments. This aged chemist, at his own expense carried out dietetic experiments on rats which led him to the conclusion that a cocoyam diet (plantain and cocoyam are the staple foods in the Ashanti Forest) caused necrosis of the liver in rats. He ascribed this damage to chronic "prussic" acid poisoning by the cocoyam in the absence of the sulphur containing amino-acid cystine. Recently HINCHWORTH *et al.* have demonstrated that hepatic necrosis is caused in rats by lack of a component of biological protein. Inclusion of the sulphur containing amino-acid methionine in the diet prevented this necrosis. Eight per cent. casein in the basic diet contains enough methionine to afford protection. I have no reference to show the ground nut content of this essential amino-acid, but the problem merits the attention of Gold Coast clinicians as pellagra is an amino-acid plus nicotinic acid deficiency and degenerative changes occur in the liver and adrenal glands. It is 16 years since WILLIAMS described this preventable disease. Very many children have died from it in the meantime.

It is hoped that these notes may arouse the interest of some of our African colleagues natives of Ashanti who may yet draw official attention to the prevalence of this deficiency or that some of my friends in Achimota College in their capacity as educationalists, may arouse socially the necessary interest.

I am, etc.

F. M. PURCELL.

Marino House

Killiney Co. Dublin, E're.

## EFFECTS OF NUTRITIONAL RETROBULBAR NEURITIS AND ASSOCIATED B DEFICIENCIES

To the Editor TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene

SIR,

Will you allow me to comment on the article by Dr REED on ocular symptoms among prisoners of war and also the discussion on Colonial nutrition and its problems, introduced by Dr PLATT

May I correct an error in Dr REED's bibliography. The date of publication of my article in the *Annals trop. Med. Parasit.* was 1934 not 1931. And in addition in 1937 after I had retired (at my own request) from the West African Medical Service I went back to Nigeria and completed some further work. This included very extensive trials with autoclaved marmite and autoclaved yeast with special reference to the ophthalmic aspect of this research.

The results of that visit were published in detail in a privately printed report (fifty copies) in July, 1938, and in a more abridged form in *J trop Med Hyg*, April, 1939

In the discussion on Colonial nutrition and its problems, one notes no references to the deficiency diseases which occurred with such frequency among the P o W in the Far East, and, incidentally, for the most part within our Colonial boundaries. Surely those experiences described as they have been in such detail, and occurring as they did under such well-defined circumstances, are invaluable, and, as Dr REED's admirable—though incomplete—survey has so well illustrated, the syndromes found there were not new, but have been shown again and again to have occurred in many parts of the world, particularly our own Colonies. Moreover, certain common factors have clearly emerged, namely, that whether precipitated by poverty or ignorance, privation or duress, captivity or detention, indifferent or incompetent dietary management, there has been demonstrated that when the dietary has consisted of such foodstuffs as sugar-cane, manioc, rice (deficient in all the "B" vitamins, and other nutrients alike) as staple foods, and there has been an insufficiency of protein, these conditions have been produced. While the need for continued investigation with further excluding trials, etc (especially being made possible in the light of still further advances in biochemical knowledge), of extended and other survey, remains obvious, it would appear—as we note also Dr DAVEY has stressed in these discussions—that many practicable considerations are open now to implement a more active policy in the prevention of these syndromes and of malnutrition generally. If one might be permitted to add to these considerations benefiting from one's own experiences in Nigeria, may I make the following suggestion. The prevalence of what I would term now the "pellagra-ariboflavinosis" syndrome with which was so commonly associated nutritional retrobulbar neuritis—was particularly marked in certain schools there (at least the severity of the visual effect, if left untreated, has been again fully emphasized by the P o W case—compare with them "the school child *listening* in school because he could no longer *see* to read") And though, no doubt, Nigeria, like all other producer countries, is experiencing a period of prosperity—but I believe, as events following the previous world war have shown, only an artificial wave of prosperity—and manifest malnutrition may have temporarily disappeared, it will come again. I suggest, then, a method by which at least we can make sure there can be no possible recurrence of these conditions in schools and institutions there. These are —

- (1) Compulsory medical inspection of all schools at least two or three times a year. This inspection to include, and have recorded, routine visual tests

- (2) Compulsory provision of a balanced dietary for all boarders whatever the class of school. Government, mission, private

- (3) Registration of weight and height of all school children preferably at the beginning and end of each term, with periodic checking. (The

use of weight recording in prisons has proved one of the most valuable checks on nutrition in Nigeria.)

(4) Provision of one free school meal a day for that class of scholar I have termed the boarder-out," i.e. who is too far away to live at home too poor to be a true boarder but who lodges with friends obtains his own food at varying intervals from his home does his own cooking, or else is subvented as a lodger—a pernicious though largely unavoidable system, and to consider this provision as a possible extension for the very poor

If a whole time schools medical service could be provided it might go a very long way with these provisions to ensure a sound nutritional standard for many of the younger generation.

I am, etc.

Cheltenham

D FITZGERALD MOORE

### MANSON AND CHALMERS MEDALS

The MANSON and CHALMERS Medals will be presented by the new PRESIDENT at the Annual General Meeting of the Society to be held at Manson House on Thursday 19th June 1947

#### Manson Medal Awards for 1941 1944 and 1947

At the Council held on 20th March, 1947 the MANSON MEDALS were awarded as follows —

- 1941 Professor EMILE BRUMPT Faculté de Médecin Paris
- 1944 Sir S. RICKARD CHRISTOPHERS, C.B.E. F.R.S. L.M.S. (ret.)
- 1947 Dr CHARLES MORLEY WENTON C.M.O. F.R.S.

#### Chalmers Medal 1947 Award

The nominations received were considered by the Council held on 20th March, 1947 and the CHALMERS GOLD MEDAL, 1947 was awarded to Dr DAVID GARNET DAVEY Biological Department, Imperial Chemical (Pharmaceuticals), Ltd., Manchester

At the Council held on 10th April, 1947 it was decided to award in 1948 the three outstanding CHALMERS MEDALS due for 1941 1943 and 1945 respectively

Any Fellow is entitled to make nomination. The proposer shall deposit with the Honorary Secretaries of the Society not later than 28th February 1948 statement which must include the following data —

- (1) Date of nominee's birth.
- (2) A detailed statement of nominee's claim for consideration, indicating the year or years in which any work mentioned was carried out.
- (3) A list of the nominee's published papers with dates.

Full conditions of award are to be found in the Year Book, p. 6.

The previous number of these Transactions, Vol 40, No 6,  
was published on May 14th 1947 ]

# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

VOL 40 No 6 JULY, 1947

ORDINARY MEETING  
of the Society held at  
Manson House, 26, Portland Place,  
on

Thursday, 20th February, 1947, at 8 p m

THE PRESIDENT,  
C M WENYON, C M G, C B E, M B, B S C, F R S,  
in the Chair

**The President** We are very fortunate to have the privilege of hearing Professor VAN HOOFF speak to us this evening on the subject of trypanosomiasis which he has made his special study for the last 30 years. Professor VAN HOOFF went out to the Belgian Congo in 1916 as a doctor in the Belgian Army. He soon left that service and entered the Administrative Department, but he had a flair for research and investigation and did not allow his official duties to interfere with the research which he carried on so successfully all those years. In 1925 he joined the International Commission organized by the League of Nations to study human trypanosomiasis and was in Uganda with this Commission for about 3 years. The investigations carried out by him and other members of that Commission were published in the *League of Nations Reports of 1927 and 1928*. Not the least interesting part of this work was that carried out by Professor VAN HOOFF on the epidemiology of sleeping sickness and the practical methods of diagnosing this disease. The work he will tell us about tonight has been carried out in the Belgian Congo. Much of it has already been published in a number of papers, but he has a good deal of new information which he will put before us this evening. He has now retired from the Belgian Congo and has taken up a professorship at the Institute of Tropical Medicine at Antwerp, but he is so wedded to his work in the Belgian Congo that he tells me that in a few weeks' time he is going to fly back to that country in order to set on foot other lines of investigation which he thinks should be carried out.

Without further introduction, I will call on Professor VAN HOOFF



# THE SECOND ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE CHADWICK LECTURE

## OBSERVATIONS ON TRYPANOSOMIASIS IN THE BELGIAN CONGO

BY

PROFESSOR L. M. J. J. VAN HOOFF, M.D.

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These observations are the result of researches made in the course of the last 16 years, together with Dr C. HENRIARD and Mlle. PEEL. I present them in the name of the team, and it is my duty to say that my co-workers have made contributions at least as important as mine to this work.

### INTRODUCTION

#### REVIEW OF THE ACTUAL EPIDEMIOLOGICAL SITUATION

While not neglecting any means for fighting the tsetse, the principal object of the Medical Service in the Congo has for many years been the sterilization of the human reservoir of trypanosomes. This policy had above all a humanitarian object—to save the greatest number of patients. But it made great demands—numerous personnel, a disciplined population, various active drugs in abundant supply, co-operation of the administrative authorities, and a spirit of sacrifice and devotion in doctors and their assistants. The method, moreover, was only valuable on the understanding that it was prosecuted efficiently in

the whole infected zone until eradication of the infection was complete. For a long while results were encouraging, as the following table proves —

TABLE 1

Year	Examined	Old cases	New cases	Percentage of new cases under examination
1926	2,145 177	50,775	24 982	1 20
1927	1,704 477	70,040	16,260	0 95
1928	2,126 356	46 972	24 440	1 16
1929	2,383,802	50,244	27,046	1 12
1930	2,779,448	70,423	33 562	1 20
1931	2 685 768	62 272	25 582	0 95
1932	2,832 083	62 272	21 346	0 75
1933	3,572 433	77,268	27 939	0 78
1934	3,824 097	93,954	24,101	0 63
1935	4 350,270	86,147	18,930	0 43
1936	5,282,046	66,774	18,708	0 36
1937	5,034,442	53,429	14,021	0 29
1938	5,034,331	50,980	13,454	0 27
1939	5,216,841	45,785	12 886	0 25
1940	4,860,096	40,510	11,837	0 24
1941	3 048,213	35 189	10,051	0 28
1942	3,256 056	35,104	9 968	0 26
1943	3 621,820	24 930	10,093	0 27
1944	3 713,347	25,251	10,142	0 27
1945	3,293 175	20,822	9,441	0 28
		18,953		

Nevertheless, since the years 1936-37, despite the increase in the personnel employed and the use of better methods of treatment, the results follow a slow curve tending to an equilibrium showing about 0 25 per cent infected among the population examined. From practically all parts of the endemic area came the statement that trypanosomes became resistant to arsenic, a fact which had only been noted previously in certain regions, especially in those where the campaign had been most intense and long established. The results of our enquiry were published in 1938 but since then the frequency of cases resistant not only to trypanamide and the other pentavalent arsenicals but also to antimony and to organic drugs had so increased that the Directorate of Medical Services believed it necessary to modify the technique of chemotherapy employed and to give chemoprophylaxis a more important position. During the war, on account of the reduction of medical personnel called in great part to the armed forces, the introduction of chemoprophylactic measures was extended by using intrapol and the diamidines (pentamidine and propamidine). The study of strains coming from endemic foci has produced evidence of the variation in virulence (or pathogenicity), drug resistance and cyclical transmissibility, and these are the three factors which have stimulated the greater part of the researches herein reviewed.

## I. VARIATIONS IN DRUG RESISTANCE.

The idea that there are trypanosomes more resistant than others to arsenicals is already old in the Belgian Congo and must not be mistaken for the recrudescence of parasites during the treatment of chronic cases. It was indicated by BRODIE (1906) and by BRODIE and RODHAIN (1908) during their first trials of stoxyl. It was observed in certain cases treated in 1920 by L. PEACHE (1921) with trypanamide, it was studied by the International Sleeping Sickness Commission of the League of Nations which reported naturally resistant strains in the valley of the Semhki (VAN HOOFF 1923) and accounted for attempts to increase artificially the resistance (DUKE, 1936).

The well-known fact that subcurative doses render a trypanosome resistant against any given drug caused anxiety to the medical authorities who had paid careful attention to the development of this characteristic. Doubtless the frequency of resistant strains appeared greater because the attention of clinicians had been called to it but in fact we had long been aware of the increase in the number of resistant strains. This increase was reported by SETROU (1933) in the Mayumbe, where it was estimated between two definite dates—1929 and 1933 by CHITTERTON (1932) in the region of Stanleyville, and by us (VAN HOOFF, HENRIARD and PEEL, 1938) in the entire colony.

While taking into account the distinction made by WARRINGTON YORKE and MURDATROYD (1935) regarding trypanosomes appearing in relapses among advanced cases, it appears beyond all doubt that we were finding in endemic and epidemic foci a more or less important proportion of strains resistant to stoxyl and trypanamide. In the second place this percentage was obviously everywhere greater in the regions where the sleeping sickness campaign had been carried on longest. It was thus quite natural to conclude that the campaign itself was responsible for the production of arsenic resistant strains by the repeated administration of subcurative doses. So much the more so in that YORKE and MURDATROYD (1935) had shown that it is extremely easy to increase in laboratory experiment the resistance of *Trypanosoma gambiense* against any given arsenical. There is, however another plausible explanation offered by BARLOVATZ (1933) the arsenical treatment might have acted selectively on the mass of strains circulating in endemic areas and spared the more resistant. As these are transmitted cyclically without modification of their resistant character it is obvious that they would become predominant. One or other of these hypotheses must considerably influence the programme of the campaign but cannot be accepted without confirmation of the facts.

Actually according to our recent observations we believe that it is the less resistant trypanosomes which are transmitted best by glossina. This explains the survival of a small proportion of these trypanosomes in the oldest foci and their predominance in new foci and in epidemics. We shall return to that later.

## 1 ORIGIN OF RESISTANT STRAINS

The fact that resistant strains have been observed since the first trials of atoxyl and from the first experiments with tryparsamide makes us believe that they have always existed in nature. Actual proof of this is difficult to furnish in the Belgian Congo where pentavalent and trivalent arsenicals are employed in the treatment of so many diseases. Nevertheless, researches were made in many new infected areas, for instance in the region of Gemena in 1934, where the influence of any previous arsenical therapy could be reckoned negligible. In these areas a small number of resistant strains were isolated.

## 2 VARIATIONS IN RESISTANCE DURING ARSENICAL TREATMENT

Although this question has long been studied by many authors, it appeared interesting to make some observations indicating the very variable behaviour of *T. gambiense* towards an arsenical drug, as well as the influence of other factors (Tables 2, 3 and 4).

In these three patients a suitable tryparsamide treatment (at least ten injections of 2 to 3 grammes, i.e., 4 to 7 cg per kg) was followed by a relapse. The trypanosomes of the relapse did not show that they had acquired any resistance. However, the trypanosome of the Patient B, after cyclical transmission through a pig, showed itself to be easily susceptible to transformation into a resistant trypanosome.

There was one chronic patient whom we followed for 15 years. After the fourth relapse in 1933 the trypanosome responded to 2 cg of tryparsamide per kg, although the patient had been treated for a long time by this drug—since the beginning of his infection, and as long as the cerebrospinal fluid had not returned to normal (Table 5).

In 1937 he was again infected. It is difficult to say whether it was a relapse or reinfection. Whichever might have been the case the trypanosomes were with difficulty transmissible and showed no tryparsamide resistance.

TABLE 2

<u>Observation Marçal</u>			
Marçal (the patient)	<u>mechanical</u> transmission →	G. p.	cured with 5 cg per kg. Tryparsamide
treated with 10 injections 5 to 7 cg per kg in 1933	cyclical transmission	↓	
		G. p. 51	cured with 2 cg per kg Tryparsamide
Relapse in 1935 (after 1½ years)	<u>mechanical</u> transmission →	G. p. I and G. p. II	
		1 cg and 2 cg	Tryparsamide
		long negative periods	
		5 cg	cures

TABLE 3

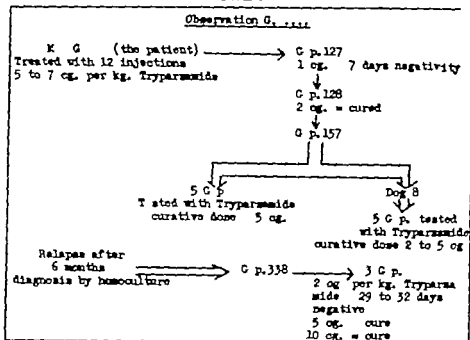


TABLE 4

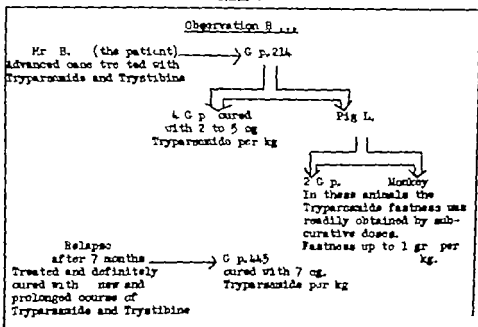
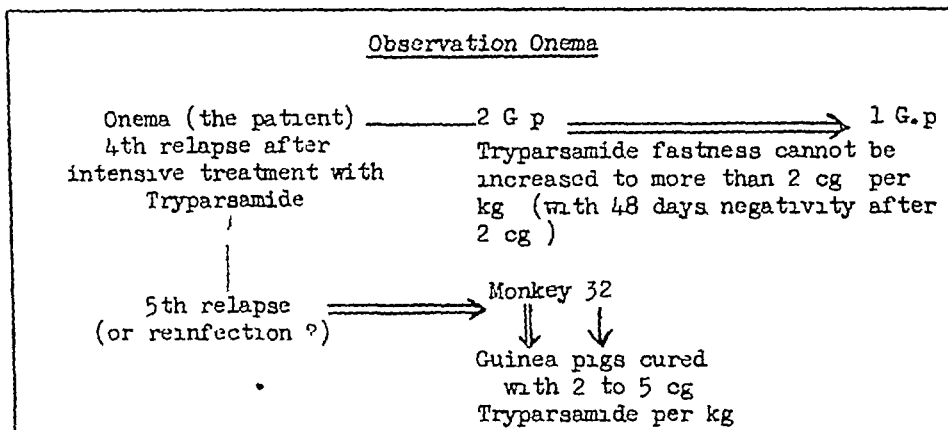
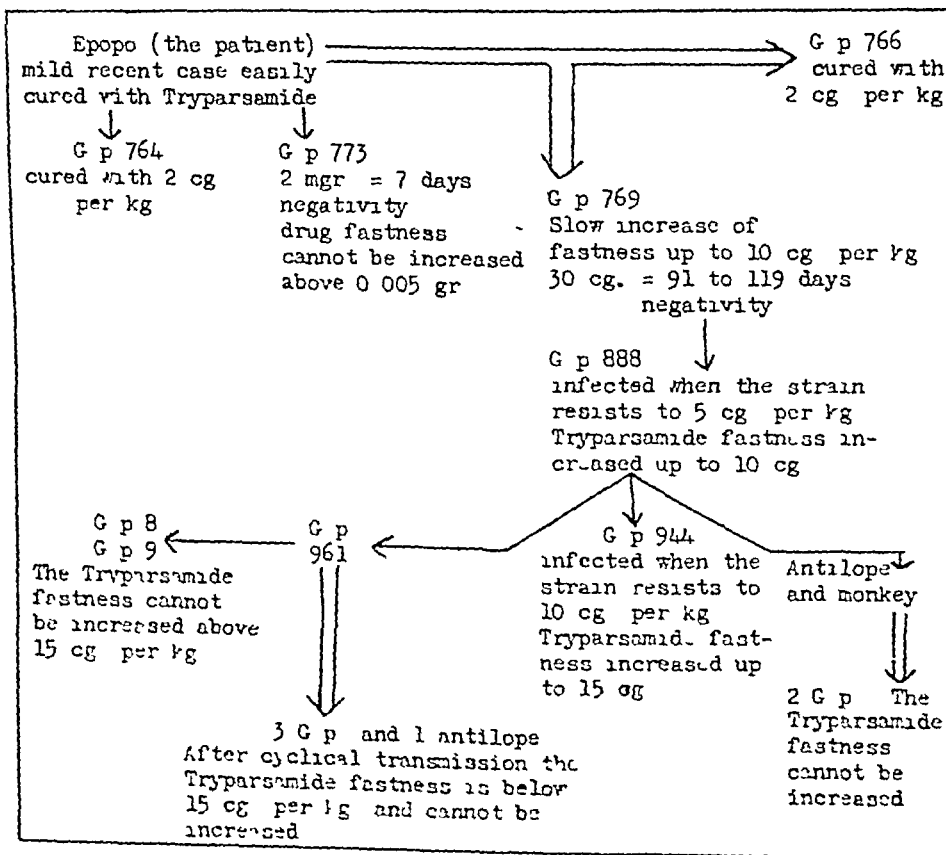


TABLE 5



These observations show that the routine treatment by tryparsamide which we practise in the Congo does not necessarily increase the resistance of the parasites. We have, moreover, quite frequently met the greatest difficulty in creating this resistance. Here in Table 6 for instance, is an example —

TABLE 6



Thus the trypanosome (strain Epopo) had been subjected for more than a year during frequent mechanical and cyclical transmissions, to subcurative treatment. Passages had been made when the strain appeared to have acquired a solid degree of resistance independent of the chronic nature of the infection. The change of host did not facilitate the increase of resistance which in the test reached the figure of 15 cg. per kg.

Frequently the creation of a resistant strain appeared to be favoured by a given host, or in other words, the trypanosome would easily lend itself to growth of resistance in one guineapig while in another infected at the same time failure was complete. In one guineapig submitted during 6 months to subcurative doses the relapse would appear after a dose of at the most, 10 cg. per kg. whereas in another the trypanumade resistance would be complete for 1 gramme per kg. after 1 month only of subcurative treatment. Thereafter when these trypanosomes are mechanically transmitted to clean pigs, they show their real degree of resistance which would be for instance, respectively 5 cg. and 50 cg. per kg.

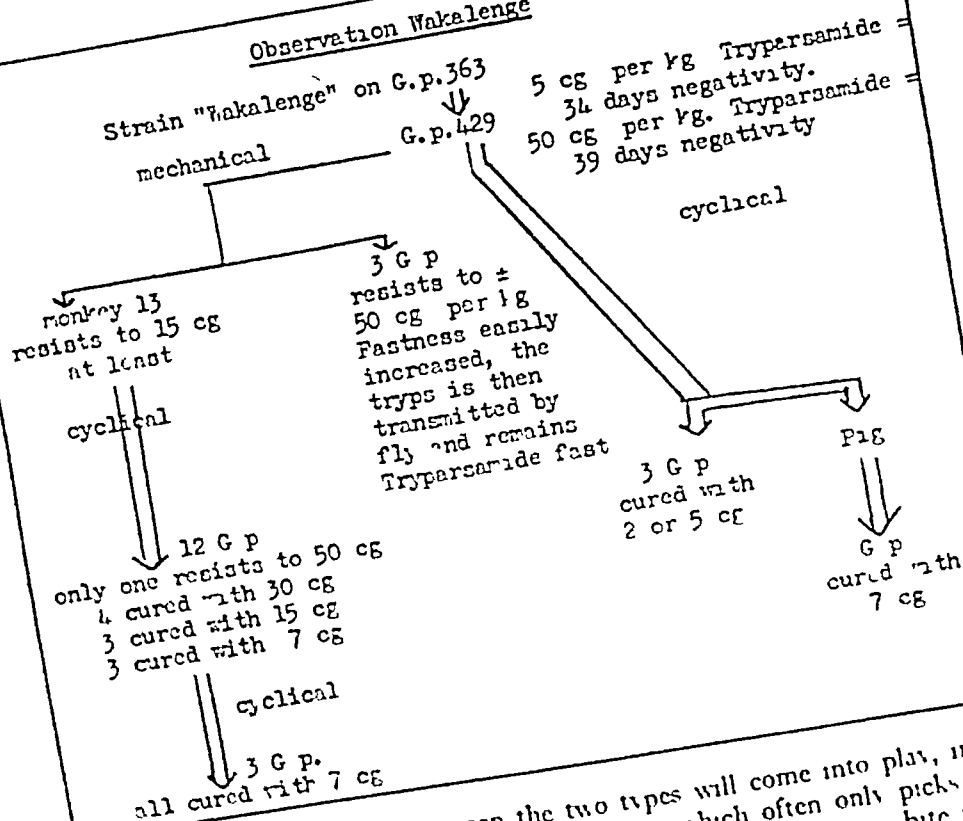
Mechanical transmission confers on a new animal a trypanosome which shows its real resistance that is to say that the rapid reappearance or the persistence of the trypanosomes in the blood can only be due to the new character of the parasites and not to the chronic nature of the infection or to localizations of the flagellates in the internal organs. There are only very feeble variations in the degree of this resistance during consecutive mechanical transmissions.

Cyclical transmission has generally the same effect, but this rule is not absolute. In this regard we feel we ought to modify our opinion on the cyclical transmissibility of the acquired character of drug resistance, and admit that it is not invariably transmitted by *Glossina* to all the animals which the fly infects. Table 7 gives an example.

Starting from Monkey 13 during cyclical transmission, the strain lost its resistance except in one guineapig. The resistance is maintained on the contrary in a parallel experiment starting with a guineapig. Moreover in cyclical transmission starting from Guineapig 429 resistance diminished to such a degree that a guineapig was cured by a dose of 2 cg. per kg. These changes did not appear to imply the influence of any special host, monkey or pig, because they could be produced without them.

These results can be interpreted in the following manner: among the mass of trypanosomes which infect the animal, there is a proportion which have become resistant owing to subcurative treatment and which have undergone mutation. This mutation creates a new characteristic which is only transmitted to a proportion of its progeny in the course of multiple divisions. The result is that resistant trypanosomes only represent a proportion of parasites and sometimes even a minority. Mechanical transmission which is effected by means of a great number of trypanosomes has a greater chance of introducing both types. Cyclical transmission, on the other hand, involves a few try

TABLE 7

Observation Wakalenge

panosomes and a selection between the two types will come into play, in the first case during the infecting feed of the tsetse which often only picks up a small number of trypanosomes and, secondly, during its infecting bite which may only transmit a few metacyclic flagellates.

Whatever may be the case, it seems that the selection made by the tsetse favours the less resistant types. In other words, it is the trypanosomes which have only a little or no resistance which are best transmitted by the fly.

Many experiments have given the impression that successive cyclical transmission assures the stabilization in trypanosomes of a uniform degree of resistance to tryparsamide.

We have already published some researches on the behaviour of *T. gambiense* in the pig. This work has been continued and the strain has been maintained in pigs during 1 year by a score of cyclical transmissions. The following table summarizes this trial.

This strain was found at the beginning of the experiment to be about the limit of curability in man by the usual tryparsamide treatment. Taking into account the toxicity of this product and its effectiveness in guinea-pigs, it can



TABLE 8.

Barbo (the patient) and following cyclical passages on pig

1st	Pig III	The G p. are cured with at least 2 or 7 cg. per kg but may relapse after 10 cg. The strain is easily made Trypanamide fast.		
2nd	IV	The G p. are cured with 5 cg. at least		
3rd	V	The resistance is about 10 cg		
4th	Can.	G p. cured with 5 to 7 cg.		
5th	6	G p.	5 to 10 cg	
6th	7	G p.	5 to 7 cg.	
7th	8	G p.	5 cg. at least.	
8th	13	G p.	7 to 10 cg.	
9th	19	G p.	5 to 10 cg	
10th	26	G p.	5 cg.	
11th	37	G p.	2 cg. at least	
12th	38	G p.	10 cg.	
13th	34	G p.	2 cg. 40 days negativity	Cured with 5 cg.
14th	41	G p.	cured with 10 cg	
15th	43	G p.	5 to 7 cg	
16th	49	G p.	7 to 10 cg	
17th	56	G p.		
18th	60	G p.		
19th	62	G p.		
20th	64	G p.	cured with 5 to 7 cg.	

be presumed that with doses of 7 cg. per kg. one can cure 80 per cent. of cases the other 40 per cent. relapsing sooner or later. Nevertheless, the strain in question presented, at the beginning of the experiment when it was first isolated, varying degrees of resistance. Following numerous cyclical transmissions, this diversity gave place to a uniformity because, as we think, it is the less resistant types which are best transmitted. The result was the establishment of a trypanosome with a less degree of resistance.

In the following observation this stabilization appears after the first cyclical passage (Table 9)

The question may be asked as to whether the trypanosomes in the deep organs present the same reaction to arsenicals as those in the peripheral blood. In this respect we compared trypanosomes in the cerebrospinal fluid with those in the blood of several advanced cases. In general, trypanamide resistance is comparable in the two trypanosomes. On the other hand trypanosomes in the cerebrospinal fluid are only occasionally transmissible by tsetse and cannot be grown in the media prepared by BRUTHAERT and HEYRARD for blood culture. Out of fifteen strains examined for this purpose only one was transmitted by tsetse, none could be cultured.

Routine treatment by trypanamide comprises generally one injection a week of 3 to 7 cg. per kg. for at least 10 weeks. It was interesting to check

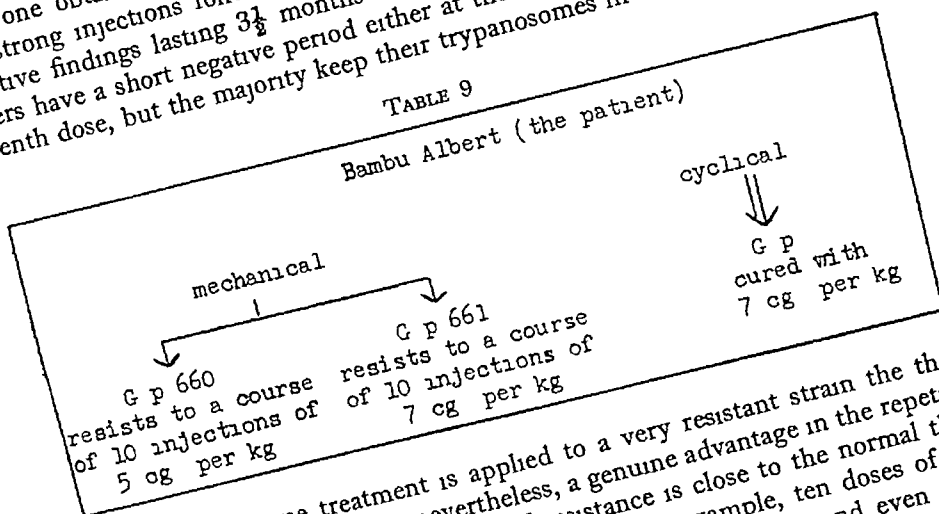
L M J J VAN HOOF

in experimental animals what would be the efficacy of these repeated doses on a more or less resistant strain and in what measure they might aggravate the resistance of the trypanosome. To this end we treated guineapigs infected with well-known trypanamide-resistant strains, either by ten equal doses or by two strong injections followed by eight medium doses. We also tried injections at short intervals, either of 3 to 4 days or daily, the results are deceptive and can be summarized thus.

When a strain resists a dose in the neighbourhood of the maximum tolerated dose, one obtains some cures (about 20 per cent) by the method of giving two strong injections followed by eight medium doses. A guineapig showed negative findings lasting  $3\frac{1}{2}$  months after such a series of injections. Many others have a short negative period either at the beginning or after the sixth or seventh dose, but the majority keep their trypanosomes in the peripheral blood.

TABLE 9

Bambu Albert (the patient)



When this same treatment is applied to a very resistant strain the therapeutic result is nil. There is, nevertheless, a genuine advantage in the repetition of doses especially where the degree of resistance is close to the normal therapeutic dose and when the infection is early. For example, ten doses of 5 cg per kg can make trypanosomes which are resistant to 7 cg and even 10 cg per kg definitely disappear.

Repeated doses at short intervals are not more effective than weekly injections.

This routine treatment does not inevitably lead to resistance in trypanosomes, nevertheless a raised resistance does manifest itself occasionally with one or another animal in an experiment, and this suggests an individual factor in the infected subject. This increase is more frequent in strains which already possess a high degree of resistance. But in applying these experiments to man treated in the same way in an endemic area, it does not seem that increase of resistance is the rule, it is rather the exception, but an exception which is an unfortunate reality, as Table 10 (page 738) shows.

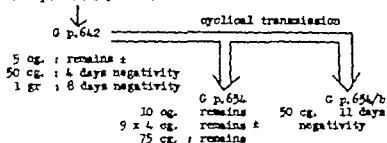
Although the trypanosome was resistant in this patient to a dose in the neighbourhood of 5 cg per kg, and it became obviously more resistant in the course of treatment, the clinical appearance of the patient was not aggravated.

TABLE 10

Mosinga Mafuta (the patient)

Recent case apparently cured with 12 x 1.75 gr Tryparsamide  
(± 5 cc. per kg.)

Relapse after 3 months



### 3 PRACTICAL CONSEQUENCES IN THE CAMPAIGN AGAINST SLEEPING SICKNESS.

The preceding observations diminish the responsibility of the medical services which have for many decades used arsenical chemotherapy in the fight against trypanosomiasis. It is probable that resistant varieties already existed. Undoubtedly we have created unknowingly and unwillingly some strains specifically resistant to stoxyl and tryparsamide and increased the resistance of some which were already refractory to the tolerated dosage. But very probably the methodical treatment of the population after careful census has brought about some selection which has allowed the survival of resistant strains. However it may be, the one ominous fact persists—that is the continuous increase of the percentage of resistant strains in the endemic zones. In that of Léopoldville, which is very familiar to us, this has reached in the course of these later years rather important figures. Before 1938 one reckoned on about 7 per cent. of trypanosomes being resistant among recently infected cases. During the war this proportion reached at least 50 per cent. and recently Mlle. PUEL has written to me from Léopoldville that the greater number of new cases admitted to hospital harbour parasites which are tryparsamide-resistant. Similar reports come from other centres. For a long time the situation has been disturbing in the Mayumbe. It is also thus in the Kwango and Kasai and above all in the Namema, where trypanosomes appear specially refractory not only to tryparsamide but also to antimonials and to Bayer 205.

The future of the campaign against sleeping sickness by sterilizing the human reservoir of trypanosomes appears seriously compromised. This so it seems to us, justifies the decision taken by the Directorate of Medical Services to extend as far as possible general chemoprophylaxis by preventive injections of Bayer 205, pentamidine and propamidine.

Already, before the war, Bayer 205 had been employed as a preventive in certain limited areas, for instance, in the "Channel," where there are about 75 per cent resistant cases

It is unnecessary to describe this method, the value of which has been well shown. The dose of 0.025 gramme per kg seems the optimum dose and one dose protects for about 3 months. We do not ignore the fact that in exceptional cases the duration of protection is shorter—a minimum of 58 days in a series of experiments which we made at Leopoldville. But the practical rule of 3 months has emerged from massive experiments in areas heavily infected, on several thousands of people subsequently observed for some years by the medical personnel in possession of a complete census of the population, which was examined by them at regular intervals. Nevertheless, "Bayerization" of endemic foci is not an easy task. It demands a census and complete examination of every individual, as well as treatment of all the cases, and finally preventive injections must be repeated every 3 months. From this point of view the protection of a population by pentamidine or propamidine presents the great practical advantage of only needing two visits a year.

The first results obtained by protective injections of pentamidine were published in 1944 and 1946 in these *TRANSACTIONS* (VAN HOOFF, HENRARD and PEEL, 1944, and VAN HOOFF, LEWILION, HENRARD and PEEL, 1946). Our laboratory trials were criticized by FULTON (1944), who remarked that the long duration of protection which we had obtained was in great part caused by a certain degree of immunity conferred on our animals and on volunteers by trypanosomes which were inoculated into them only a little while after administration of pentamidine. This objection obliged us to recommence our experiments in order to avoid any possibility of creating an immunity to trypanosomes. The results obtained on volunteers were satisfactory and we believe that we can maintain the thesis that one dose of pentamidine can protect a man for 6 months (VAN HOOFF, HENRARD and PEEL, 1946). We have learned subsequently that the preventive action of diamidino-stilbene, diphenoxypropane and diphenoxypentane had already been shown by LAUNOY and LAGODSKY in 1940.

Concerning propamidine, therapeutic trials from the curative as well as from the preventive point of view in trypanosomiasis have proved to us that this product possesses exactly the same action as pentamidine. Our results with propamidine will be published later on. They will make mention of certain toxic reactions caused by this drug. This toxicity was unknown to us when, convinced of the preventive value of propamidine against trypanosomiasis, the Directorate of Medical Services purchased a quantity sufficient to make experiments on a large scale in several sleeping sickness areas in the Belgian Congo.

A trial limited to pentamidine, started in 1942, has protected 721 natives in a focus where previous examination had revealed about 9 per cent of new cases.

Since then pentamidine and propamidine were distributed to doctors in charge of the supervision of important areas, in proportion to the available stock and personnel. These doctors were directed to draw up a report on their results and publish it. Our stocks of pentamidine and propamidine were enough to protect about 30 000 people for 1 year on the supposition that the average weight was about 40 kg and raising to 5 mg per kg the protective injection owing to the fact that we were using the isethionate instead of the hydrochloride.

Among the results received, we quote the following —

H. CLAIBORN (1946). 159 natives protected with propamidine in very highly infected area. One case positive 6 months after the protective injection (Mardena).

H. CLAIBORN. 2,835 natives in the Alencara with propamidine.

Exact results not yet to hand but generally good.

W. EDWARDS (1947). Using propamidine in the Muzengo area with new infection rate of — per cent. and in the Mwele-Zembwa area with an infection rate of 3.63 and 5.97 per cent. with certain points reaching from 28 to 49 per cent. with frequent trypanosomide resistant cases amounting to as many as 100 per cent. in certain villages. The author gave two injections at 6-monthly intervals to total of 8,000 people and watched them during 18 months without finding single new case. This report has not yet been published.

In Kongo, 5,000 people had been protected with pentamidine isethionate since November 1945. Not single case was detected after 1 year observation, although many infections occurred amongst the controls.

In various foci of the Equatorial Province (Coquilhatville) 6 000 natives got preventive injections of pentamidine. Other natives were protected with antypol and third group was kept as control. Amongst the people injected with pentamidine, two new cases occurred before the end of the first period. In the group of controls, the rate of new infections remained between 1.3 and 2.3 per cent.

There are at present 50 000 natives under observation after protection with pentamidine and propamidine, and the Medical Service has bought new supply of pentamidine which will allow us to extend the experiment to 175 000 more natives in 1947.

It has been recommended that propamidine and pentamidine isethionate should be given in 1/25 solution by intramuscular injection. Toxic reactions practically never occur with pentamidine on the contrary it seems that the same dose of propamidine sometimes causes abortion in pregnant women. For this reason we prefer in the future to use pentamidine.

One must not, however be under any illusion or attribute to the diamidines an absolute preventive value. In the first place, a few people can escape the attention of the examiners before the first preventive injection is given. A dose of 5 mg per kg. is not always curative even in the earliest stage and those cases diagnosed 6 months later may throw discredit on the efficacy of the diamidines. On the other hand, it is possible that with some people more rapid elimination or transformation of the drug shortens the duration of protection. We have observed such individual variations with Bayer 205.

However this may be, we believe that the general use of preventive drugs Bayer 205 and more especially the diamidines, is the most effective method we

Since writing this I have received news from Léopoldville on the results obtained in other areas.

possess at present for fighting the endemic trypanosomiasis complicated by arsenic-resistance. We have had proof that this method, when applied by adequate personnel with the necessary care, can bring about a complete eradication of the circulating parasite in man within 18 months, in a limited area. But other questions present themselves. Can a cleaned-up area where contact between man and the tsetse persists again be reinfected either by trypanosomes brought in from outside or from animal reservoirs of trypanosomes?

With regard to this we are able to quote a reassuring incident in the endemic area of Lake Albert, with about 150,000 inhabitants, where in 1923 there were still 5 per cent new infections annually. Complete eradication of sleeping sickness was obtained by systematic treatment of all the patients. For 8 years this area has defended itself against external or internal attack. It will doubtless therefore be possible to obtain a similar result much more quickly by preventive injections.

One cannot, nevertheless, underestimate the importance of the animal reservoir of trypanosomiasis to which we will return later.

The fate of patients, many of whom carry resistant trypanosomes, deserves some mention. In 1938 we suggested a rough test sufficiently practical to show trypanamide-resistance in each new case diagnosed (VAN HOOFF *et al*, 1938). It consists in examination of the blood and lymph gland juice 48 hours after the maximum tolerated dose. In fact, this test when positive invariably denotes a strong resistance. Controls on guineapigs often show resistance 5 cg and even 1 gramme per kg.

On the contrary, a negative result of this test in the patient does not always warrant the certain conclusion that trypanosomes would respond eventually to treatment.

The method was put into practice in the Congo from 1938 onwards, and it has been confirmed that when it is negative it is necessary nevertheless to control blood and gland juice during the course of treatment and after the last injection, for the trypanosomes can reappear later on when their resistance is slightly greater than that to the normal dose.

The result is also that when resistance to pentavalent arsenicals is found frequently in certain areas it will be advantageous to treat uniformly all early infections (with a normal cerebrospinal fluid) by Bayer 205 or by any other product effective against arsenic-resistant trypanosomes. For advanced cases it is important to determine the trypanamide-resistance in order to benefit by the most effective treatment those who carry known resistant trypanosomes against their nervous involvement, and to cause the disappearance from the blood of others of the resistant trypanosomes which the fly can still transmit.

With regard to this we have had the opportunity of trying new arsenical preparations, principally melarsen oxide and p-arsenoso-phenylbutyric acid. In collaboration with H. EAGLE, p-arsenoso-phenylbutyric acid was used both in the laboratory and on the field. This arsenical is active against trypanosomes resistant to trypanamide and the ordinary pentavalent arsenicals. It is possible

but rather difficult to render trypanosomes resistant to this product. The *p*-arsenoso-phenylbutyric acid is quite non-toxic in ordinary therapeutic doses of about 0.0004 gramme per kg. and even 0.001 gramme per kg. Daily injections are well borne and one obtains apparent cure in the majority of early cases by fourteen or fifteen injections, that is to say in 2 weeks, which is a very real practical advantage in any trypanosomiasis campaign. Unfortunately this *p*-arsenoso-phenylbutyric acid is ineffective in advanced cases with altered cerebrospinal fluid.

Melarsen of FRIEDHEIM is a product too toxic for man, and it should be definitely abandoned. On the other hand, melarsen oxide is better tolerated. It is given either by mouth in doses of 150 mg. daily for an adult, or by intravenous injection of 25 mg. a day or on alternate days. Generally a series of fifteen injections can be given without serious toxic effect. Trypanosomes resistant to trypanamide respond to melarsen oxide. Moreover changes in the cerebrospinal fluid are favourably influenced although the improvement may not be as complete as that which is obtained by trypanamide. It remains, nevertheless, true that melarsen oxide is one of the rare products which is effective in advanced cases of sleeping sickness with trypanosomes resistant to trypanamide. We need only mention in comparison certain antimonials for example, trisbiline and the diamudines, in which the action is uncertain and variable.

It has been considered earlier in this paper that trypanosomes resistant to arsenic probably existed in nature well before subcurative treatment could have produced this mutation in the parasites. We believe the same is the case with strains resistant to tartar emetic and in others resistant to Bayer 205. These drug resistances are much rarer however though they nevertheless exist even in areas where trypanamide resistance is rarely observed. They are, however more common in regions where many arsenic-resistant cases exist and this confirms the conclusions of FULTON and WARRINGTON YORKE (1941) on the necessity of preparing tartar emetic resistant strains starting from those which are already atoxyl-resistant. With regard to this, CLARKE (observations not yet published) reports from the Maniema early and advanced cases in which trypanosomes do not disappear after one or more injections of 10 cg. of tartar emetic. In many areas in the Congo we have collected strains which resist 4 cg. of Bayer per kg. Moreover among the first clinical trials of Bayer 205 in 1922-23 by VAN DEN BRANDEN and VAN HOOFF (1924), early relapses were observed after a total of 3.5 grammes in adults.

We have not yet been able to verify that the character of natural resistance to tartar emetic is maintained through cyclical transmission by tsetse. Concerning resistance to Bayer 205 we have noted the progressive attenuation of this character during cyclical transmission of a strain rendered resistant in the laboratory (VAN HOOFF, HENRIARD and PEEL, 1938). Actually however we are in possession of a strain naturally resistant to 4 cg. per kg. which remains invariable after months of passage through tsetse.

## II VARIATIONS IN VIRULENCE AND PATHOGENICITY FOR MAN AND ANIMALS

### (1) BEHAVIOUR OF *T. gambiense* IN ANIMALS

The animal reservoir of *T. gambiense* acquires a special importance when one wants to obtain complete and final eradication of human trypanosomiasis. The possibility of survival of *T. gambiense* by a chain of transmissions from fly to domestic animals is no longer doubted by us since we have observed natural infection of a dog and since we have transmitted and kept by flies on animals several strains whose infectivity for man remained unimpaired.

Certain observations relating to this question are summarized below

#### (a) Role of pigs

We published in 1940 results of ten clinical passages of *T. gambiense* through pigs (VAN HOOF, HENRARD and PEEL, 1940b). As has already been said, these experiments continued until the natural extinction of the infection by loss of cyclical transmission at the twentieth passage. Table 11 below summarizes this experiment which lasted 4 years.

TABLE 11

		Transmission Index
Mambo P. (the patient)	.	4,9
Pig III	.	6,29
Pig IV	.	6,48 -- 4,6
Pig V	.	6,46 -- 3,49
Pig Camille	↓	4
Pambu (volunteer)	↓	2,33 after 7 months
Pig 6	...	4,25
Pig 7	.	0,86 -- 0,35
Pig 8	.	0,9 -- 0,55
Pig 13	.	2,08
Pig 19	.	2,68 -- 2,53
Pig 26	.	0,72 -- 2,29
Pig 32	↓	3,41
Matadi Paul (volunteer)	↓	1,36
Pig 38	↓	3,35
Pig 34	.	2,57
Pig 41	.	2,54
Pig 43	.	4,69
Pig 49	.	0,5 -- 5,21
Pig 56	.	3,33
Pig 60	.	1,85
Pig 62	.	2,33
Pig 64	.	4,4
		0



The cyclical transmissibility remained high, right up to the last but-one passage, but diminished progressively with the later pigs in the series. In other words the pig is only a source of infection during a shorter and shorter time as the experiment proceeds and at last the transmissibility disappears suddenly at the last passage, despite the persistence of trypanosomes in the blood.

*T. gambiense* can maintain itself for many years in pigs. It causes an unrecognized condition and there is a striking contrast between the rarity of the flagellates circulating in its blood and the high degree of the transmission index during the first passages. It is not astonishing that natural infection of the pig by *T. gambiense* has not yet been demonstrated considering the difficulty of diagnosis which we have often only been able to establish by blood culture or xenodiagnosis. We have also observed that the reinfection of a pig which has become naturally cured is obtained with difficulty and even in successful cases is of very short duration. The result is that in nature one would have to look for a natural infection in endemic areas, preferably in young animals.

### (b) Role of goats

A Bolikanda strain was transmitted by tsetse from the patient to a goat and successively to a series of goats. Here is the summary of the experiment.

TABLE 12.

		<u>Transmission Index.</u>		
Bolikanda (the patient)	First stage of infection 9 97	Later		
(1) Goat 63	5 8	1,68	0 36	
(2) Goat 65	6      2 97      1 8	1 37    2 73    2 07 and 4,11 after 11 months.		
(3) Goat 69	2 24	0 35    0 34    0 after 1 year		
Goat 71	0 71    1 24	0 98    aft r 10 months.		
Goat 70	3 96    0 64			
Goat 72	2 63    2 9			
Goat 73	4,51    0 6 fto 2 months			
Goat 74	1 6			
Goat 77	4,01			
Goat 80 was infected	Malonga (volunteer) was infected			
-----				
(1)	Hemoculture still positive after 13 months.			
(2)	After 15 months the trypanosomes can still be found scarce in the peripheral blood. The transmission index is then negative			
(3)	Transmission index negative after 15 months			



In the various stages of this chain of passages six guinea-pigs were infected and the transmission index varied from 0.6 to 4.48, which corresponds to the average figures of *T. gambiense*. After a whole year of passage through goats the trypanosomes infected successively two dogs, then a pig and finally a volunteer—that is a total of 18 months and ten cyclical passages between the transference from the original case to the volunteer. It retained completely its infectivity its transmissibility and its resistance to trypanamide.

(d) *Role of antelopes.*

It has been conclusively shown by many others, notably by DUKER and COMBES that antelopes can harbour human trypanosomes and maintain their pathogenicity and powers of cyclical transmission. This latter is sometimes increased.

Concerning *T. gambiense* in the Congo this fact has been verified for antelopes of the genus *Cephalophus*. In one of these animals, the transmission index was raised to 7. Infection shows no symptoms and is of long duration. The last blood culture made after 2 years was positive.

(2) VARIATIONS IN VIRULENCE AND PATHOGENICITY IN MAN.

We published in 1933 (VAN HOOFF, HENRIARD and PEEL) the fact of extreme variations in the pathogenicity of *T. gambiense* in the Congo. After LESTER (1933) working on *T. gambiense* in Nigeria, we propose to classify the strains in three categories—

(a) Trypanosomes of low virulence which are found to be in the majority in new foci or in epidemic extensions from old areas. They are generally easily transmitted by tsetse. In man they produce an affection of slow development and trypanosomes are not numerous in the blood. Enlargement of the lymphatic glands is produced late, as are also alterations in the cerebrospinal fluid. Trypanamide resistance is rare. We have recently come across this type of trypanosome in an epidemic in the Kwango (Mazengele and Kidima) and we kept under observation without any treatment a dozen patients who during 6 months showed no clinical signs of progress of the disease. In these patients trypanosomes were only discoverable in the blood about once in the week with negative periods for as long as 3 weeks. Nevertheless tsetse were able to become infected from them as easily as from a pig during its symptomless infection. Indices up to 5.18, but very variable, were recorded in negative periods. There was no clinical symptom, no fever, no gland enlargement and no alteration of cerebrospinal fluid. Guinea-pigs infected from these strains of low virulence showed a scanty blood infection with long negative periods and survived for at least 1 year. These trypanosomes seem adapted to rapid and widespread extension among the native population even if this is scattered. It would not be surprising if some of these infections ended in spontaneous cure.

(b) Strains of medium virulence which killed a guineapig in 3 to 4 months by heavy blood infection during the latter weeks. The curative dose of trypanamide is often the ordinary therapeutic dose. In man all degrees of trypanamide-resistance are found although the majority respond to tolerated dosage. The clinical course of this affection, though not malignant, is characterized by frequent relapses and by the appearance of altered cerebrospinal fluid, 6 months or a year after the infective bite. We considered that methodical arsenical treatment in such an infected area favours a selection of resistant strains which persist and keep up an index of between 1 and 5 per cent new cases.

(c) Very virulent trypanosomes are met with sporadically in all endemic areas. These are those which recall in man and the guineapig, the evolution of *T. rhodesiense*, by the heavy blood infection, and the rapid clinical progress of the disease. The guineapig is heavily infected from the start and dies at the end of a month. In man nervous changes are evident often after 2 or 3 months. In cases which we have observed the trypanosome is very resistant to trypanamide even up to 1 gramme per kg. and is only feebly or not at all capable of fly transmission, especially in the case of relapse after treatment. It seems to us that these two factors—high drug resistance and pathogenicity on the one hand and arsenical treatment on the other—combine to modify the power of cyclical transmission in the tsetse.

### (3) *T. brucei* AND ITS ADAPTABILITY TO MAN

The great variations in the virulence of *T. gambiense* have suggested examinations of strains of *T. brucei* collected in areas where sleeping sickness was active. Three strains from natural infections, a dog, a pig and a goat respectively, served for the different experiments summarized below.

(a) *Strain from a Dog (Thysville)*—A natural infection of a dog which died 4 days after its arrival in the laboratory suffering from characteristic keratitis.

(b) *Strain from a Pig (Kingandu)*—Natural infection of a domestic pig, complicated by *T. congolense*. An exceptional case of natural infection of the pig by *T. brucei*, which is worthy of note.

(c) *Strain from a Goat (Iokambe)*—Natural mixed infection, *T. congolense* and *T. brucei*.

The *T. brucei* were transmitted by tsetse to a series of pigs, goats, monkeys, guineapigs and dogs. We also tried to infect human volunteers.

In pigs *T. brucei* produces a quite benign infection with little or no symptoms. Trypanosomes do not multiply actively as in the guineapig and infection remains quiescent for 4 to 5 months. Following this, it becomes almost unnoticeable. The blood is only positive at rare intervals and blood culture of xenodiagnosis is necessary to control spontaneous cure, which happens between 1 year and 15 months later. Once cured, the pig becomes refractory

to infection with *T. gambiense* but the pig cured after an infection with *T. gambiense* can be infected with *T. brucei*. The transmissibility index of *T. brucei* can reach the figure of 12 but generally hovers about 5. After all, infection by *T. brucei* does not differ from that by *T. gambiense* except that it is a little more acute and flourishing.

In the goat *T. brucei* produces a serious affection, fatal in 3 to 5 months, bringing about brain lesions and alterations in the cerebrospinal fluid, keratitis and unsteady gait. Trypanosomes are constantly present in the blood and the transmission index averaging 6 to 8 can rise as high as 29.

The dog undergoes the classical acute infection so well known. The transmission index is from 4 to 5.

Many monkeys were shown to be refractory both to mechanical infection and to the bite of tsetse. The monkey *Cercopithecus galentus agilis* is infected with difficulty. But as in the case of *T. gambiense* it exercises an influence on the power of trypanosome transmission the index of which was raised up to 48-57 by the Thysville strain. Trypanamide-resistance in these types of *T. brucei* is about 30 cc. per kg. Between 2 and 10 c.c. of normal human serum are necessary to cause a fleeting disappearance of flagellates from the peripheral blood, which can, however, be rendered serum-resistant.

Seven volunteers submitted themselves to the bites of tsetse infected with *T. brucei* none contracted trypanosomiasis even with the trypanosome which had become serum-resistant, but we noted a curious fact with one of them who had been bitten, probably on 4th October 1938, by a single infective tsetse, and was found positive on 25th October but since that time negative. He had not, moreover, shown any clinical sign of infection.

On the whole the *T. brucei* in our endemic sleeping sickness areas did not seem to have any particular characteristic. Their rarity seems to correspond with the rareness of game: the pig is capable of playing the role of a healthy carrier. It was not possible to show that it could become pathogenic to man.

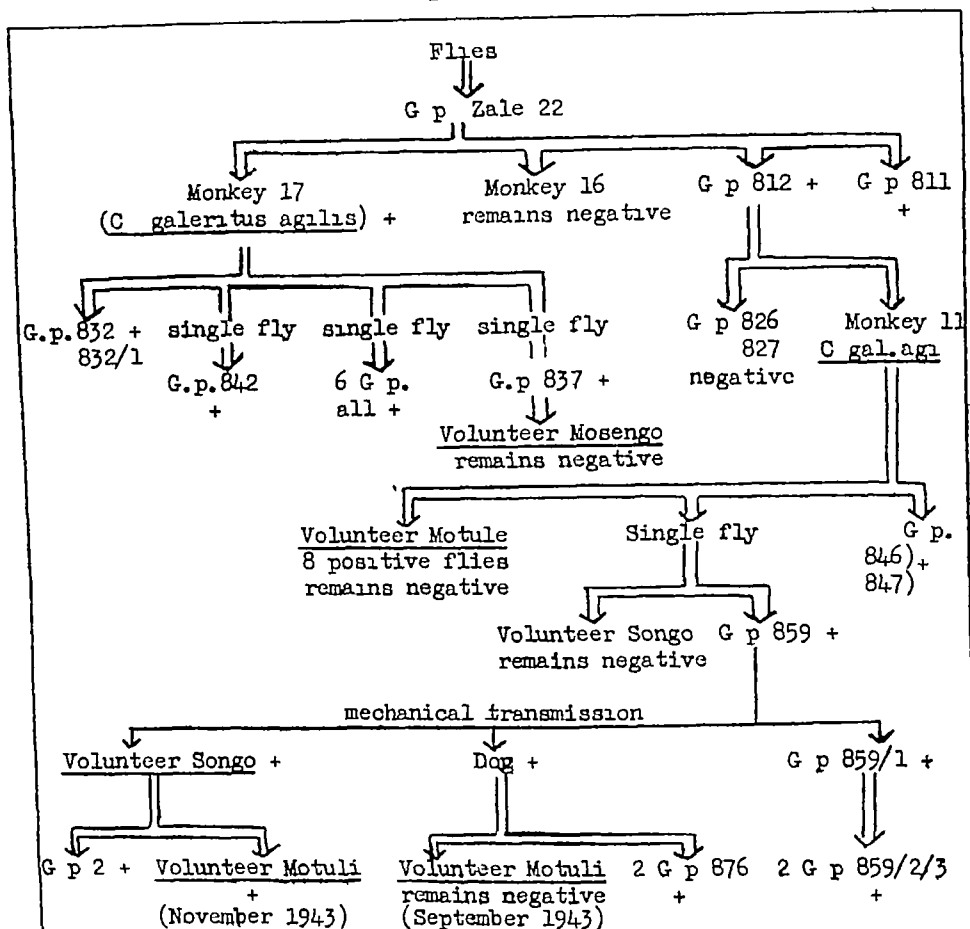
#### (4) *T. gambiense* IN TSETSE.

It remained also to examine the behaviour of *T. gambiense* collected from wild infected tsetse.

On the whole this *T. gambiense* did not differ from those we collected from patients. They are more or less transmissible and some are trypanamide-resistant. We ought, however, to mention one strain which behaved in a very peculiar manner: the strain Zale 22. It was obtained by feeding on guinea-pig wild flies captured at a site near Léopoldville, close to a native village, and crossed by well-frequented paths.

The scheme given in Table 14 summarizes the different passages made with this strain —

TABLE 14



This strain, Zale 22, transmissible to the monkey *C. galeritus agilis* and to the guinea pig by infected tsetse, could not be established in three volunteers. The infection of a volunteer succeeded only after mechanical transmission and the massive inoculation of infected guinea pig blood into him, and from then onwards this strain could be transmitted cyclically to a second volunteer. The introduction of a dog into the chain of mechanical transmissions did not produce this effect, it was necessary first to succeed in mechanical transmission to man in order to render the trypanosome cyclically transmissible to man. However this strain, Zale 22, is only moderately resistant to trypanamide (about 20 cg per kg). Its feeble pathogenicity for the dog and its appearance when in the salivary glands rules out the hypothesis that it may be *T. brucei*.

We believe we have seen in this experiment proof that the infectivity of *T. gambiense*, transmitted by tsetse for any given host, can be lost in the course

of its adaption to one or many different hosts. We have already noted one analogous fact—the strain which for a long time had been maintained in man and monkey became unsuitable to the guinea pig (Strain Onema, VAN HOOFF HENRARD and PEEL, 1940c.) With regard to trypanosome Zale 22, it seems to us possible that it underwent modification during its passage through domestic or wild animals, although our experiments on pigs, goats and dogs, probably too limited, did not seem to confirm this hypothesis. But in our transmission series through domestic animals each passage was effected soon after the infection in each animal. It would be after long maintenance, perhaps in a single host, that the trypanosome would become adapted to this host, and much less adapted to another.

#### (5) OBSERVATIONS ON IMMUNITY

Our trials on the preventive action of Bayer 205 (VAN HOOFF HENRARD and PEEL, 1940a) left us with the impression that trypanosome antigen did not produce any immunity sufficient to prolong the protective period which followed the injection. It was for that reason we omitted this factor during our experiments with pentamidine. Following the criticism of FULTON, we recommenced our experiments and were forced to recognize that a certain immunity is produced by liberation of antigens from trypanosomes injected and destroyed during the period when the drug is exercising its trypanocidal activity. This immunity is however of short duration. In man and in animals cured by therapeutic agents which do not persist in the body reinfection succeeds almost immediately. In the monkey *C. galerrus agilis* we observed, (VAN HOOFF HENRARD and PEEL, 1937) a solid but rather inconstant type of immunity. On the contrary in the pig, even after spontaneous cure, reinfection is possible although of short duration, by another strain or by that used for the first infection.

These facts are confirmed by experiments (not yet published) made on our strains of *T. gambiense* by BRUTHAERT and HENRARD on the culture of trypanosomes. The blood of an untreated case used in the culture medium prevents the multiplication of trypanosomes which have already been cultured. This inhibiting action disappears as soon as the patient is cured or apparently cured. In the same way trypanosome culture does not succeed in a medium when blood from the patient himself is employed in its preparation. It is noteworthy that in blood culture the blood of an animal carrier of *T. rhodesianus* or *T. brucei* prevents the growth of *T. gambiense* and inversely although *T. congolense* grows in the blood of these animals and of man.

The degree of trypanamide-resistance has provided a method of identification of *T. gambiense* strains and suggested a simple experiment. Guinea pigs are infected by a non-resistant strain. When the infection is well established and immunity has had time to develop from several crises of trypanolysis such as always take place at the onset of the disease, they are then infected again

with a resistant strain. If, following this, an ordinary dose of tryparsamide cures the animals, one readily supposes that the inoculation of the resistant strain has not been successful and has been prevented by the immunity caused by the first infection. This experiment succeeded perfectly with certain strains of *T. gambiense* and failed with others. In our experiment the immunity was complete or nil for all the inoculated animals, which goes to prove that immunity varies according to the antigen and not according to the individual reaction. These experiments are still going on with the purpose of finding out if the immunity conferred by the antigenic action of trypanosomes can reinforce the action of trypanocidal drugs against resistant strains.

### III VARIATIONS IN CYCLICAL TRANSMISSION

There is little we can add to the very extensive researches on this question, particularly those of DUKE and CORSON.

It has already been shown that the transmissibility index varies widely in the patients at the moment of diagnosis. But in a given man, as in animals, it reaches the maximum at the height of infection in the first period of the disease and diminishes subsequently to nil when the patient passes into the chronic stage. In certain animals, especially in *C. galeritus agilis*, it can reach a very high figure. Cyclical transmissions seems to vary along with the ability to grow in culture, and the blood of *Cercocbus* which considerably raises the index also favours the artificial culture of the parasites (Experiments of HENRARD and PEEL, not published).

The abundance of flagellates in the blood at the time of feeding tsetse has only a relative influence for one strain and any given host. It does not allow one to forecast the index of transmission and this remains very high with a pig even when diagnosis cannot be made except by culture, and it can be nil in guineapigs highly infected with a strain which cannot be transmitted. The more the disease develops into the chronic stage with grave clinical symptoms, the less the trypanosomes can be transmitted. Trypanosomes in the cerebrospinal fluid can only rarely be transmitted.

When the trypanosome stays for a long time in the pig in the course of frequent cyclical transmission, the power of transmission only persists for a shorter and shorter time and can fall away rapidly. The same phenomenon is observed in guineapigs and is probably produced in man where one sometimes finds in recent cases trypanosomes which the tsetse can no longer transmit. It has been shown in an experiment that one strain of *T. gambiense* collected in wild tsetse did not succeed in infecting three volunteers by cyclical transmission.

One is forced to suppose that the change of host during the acute stage of the disease favours the maintenance of the power of transmission and of the virulence for certain hosts, and particularly for man. In the course of cyclical fly transmission through different mammals the mutation of trypanosomes can



occur. We do not know if drug resistance appears in these conditions with certain trypanosomes, but some observations have suggested it. In others we have seen that the trypanosomes after one cyclical passage in an animal, become sensitive to subcurative doses and easily acquire high resistance. On the other hand, cyclical passages are more easily effected with non-resistant strains and bring about a kind of selection in the mixture of more or less resistant parasites carried by the vertebrate host.

Finally the strains and the flies at our disposal have allowed us to estimate the importance of the short forms in cyclical transmission of trypanosomes according to the hypothesis of M. ROBERTSON (1912, 1913). Our experiments were made on man, guinea-pigs and the monkey *C. galentus agilis*. This monkey was well adapted to the experiment on account of the high proportion of salivary gland infection in flies fed on it. A box of new flies which had never fed before was placed daily on each man or animal in which the percentage of short and long forms in the blood was estimated daily. The experiment in man lasted 24 days and necessitated the use of 1 048 tsetse—that on the guinea-pig 23 weeks using 6,818 tsetse, and on the monkey 28 days using 991 tsetse. Curves showing the variation between long, medium and short forms compared with the transmission index did not show in any case any influence of the morphology of the trypanosome on its aptness to develop in the tsetse. On the other hand, they showed clearly that for each man or given animal the index is higher when the flagellates are abundant in the blood, which corresponds to the period of maximum multiplication preceding the trypanolytic crisis.

In *Cercocerus* it is in reality the number of trypanosomes in the peripheral blood which determines the variations in the curve of the transmission index. However trypanosomes never swarm in the blood of *Cercocerus* as they do in that of the guinea-pig. It is during the transmissions from the monkey that the greater frequency of salivary gland infection in males than in females is most marked—32·4 per cent. male against 21·4 per cent. females.

We have shown elsewhere that tsetse have become infected in greater numbers when the infecting feed has not been preceded by any other feed. We can add that the infection of flies does not undergo any seasonal change in the climate of the Congo.

#### SUMMARY

The resistance of *T. gambiense* to pentavalent arsenicals especially to trypanamide, is not due only to the action of subcurative doses—it can exist as a natural characteristic of certain strains. This resistance is often difficult to create in the laboratory and the routine treatment given to patients does not inevitably increase it in the case of a relapse.

If its frequency increases in an endemic area it is rather because the treatment has removed a large number of non-resistant strains from the stock of circulating trypanosomes.



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## DISCUSSION

## DISCUSSION

Dr C C Chesterman It is a great privilege to open the discussion and pay a tribute to my old friend Dr VAN HOOF His lecture has been both understandable and inspiring He could have made it in any one of five different languages, but he has given it extraordinarily well in our mother tongue The saying goes that in the Congo a tribe of pygmies has done the work of giants—the reference being only to the small number of the Belgians As you realize, they have achieved outstanding success with great energy and enterprise, and with method and continuity in their work The table showing statistics during the last 20 years reveals that 10 years ago they were examining with the help of their subordinate staff nearly half the Congo population annually, a figure I have never heard of as being equalled in any other colony In spite of the disappointment of not being able to reduce the endemicity below 0.25 per cent, it has been possible to eradicate sleeping sickness in some areas by regular examination and treatment of the people After many years those areas have not been reinfected either from outside or from within, which gives great hope that even with existing methods the disease can be got under control Dr VAN HOOF has spoken a good deal about resistance the first resistance that one meets with in dealing with primitive populations is from the natives themselves I remember that the first patient I treated went home to her village and was bludgeoned to death for coming to us instead of to the witch doctor Some time after, during a tour of inspection, I found the old Chief who was responsible He committed the further crime of hiding two of his wives who had the disease Now that I have left the Congo I can state that I got my own back, it was still in those days permissible to use the chicotte, and the Chief got the benefit of it At one time endemicity was 30 per cent, but after 8 years, merely by continuous treatment of all infected cases and regular examination at first four times a year, then twice a year and finally once a year, the area was completely freed from the disease and has remained so for the last 15 years One is always discouraged in a new area at finding the first cases responding well to treatment, but later on getting resistant cases It is good to realize the explanation given us and to feel that nature is on our side she does not condemn us for stepping in where angels fear to tread, she limits the transmission of resistant strains, the more resistant are less easily transmissible I hesitate to speak about the scientific side of the work having been so long away from it, but it is a great gain to know that there are drugs which can be given by mouth It is also a great relief to realize that there are new drugs which can be used when you have a second stage case entirely resistant to tryparsamide, melarsen oxide is to be welcomed as a second string to one's bow One is always afraid when cleaning up these primitive areas of population that we are putting a rod in pickle for ourselves For the last 50 years 100,000 people have been dying annually from sleeping sickness in Central Africa, but the number has been greatly reduced, and it is

possible we may be able to reduce the percentage dying from malaria by drugs like paludrine. We shall soon see population pressure in Africa as it is developing in India. It means that while we are doing a job like this there must be an all round amelioration of the lot of the people with social and economic development keeping pace with hygiene. In the Belgian Congo there is a planned economy. Although the natives are given a great deal of freedom their lives are directed into what seem to be useful channels and occupations and one hopes to see developed a people with a balanced economy superintended by a benevolent government. It is men like Dr VAN HOOFF who have inspired a great deal of this progress in the Congo and we are honoured indeed to have him here tonight.

Dr D. G. Davey. I should be grateful if Prof. VAN HOOFF would clear up one point for me. I believe it is usual in cases of trypanosomiasis even in the very early ones to give courses of drug extending over a fortnight or longer. I presume such an extended treatment is considered necessary to achieve as many cures as possible. In prophylaxis, on the other hand, it appears that only one injection of drug is given, and this a smaller quantity than for curative treatment, and it is hoped that protection against infection with trypanosomes will then last for 6 months. If indeed, this happens it is quite the reverse of what happens in laboratory infections. In them, to achieve a prophylactic effect lasting for only a few weeks it is necessary to give a dose of drug usually several times bigger than that necessary to cure an established infection. Consequently I cannot help wondering if prophylaxis of human trypanosomiasis is really complete. Is it not possible that single doses of drug, spaced at such wide intervals may serve only to mask early infections rather than eradicate them, and may these infections not become manifest later as cases in which the nervous system is involved?

Dr C. A. Hoare. I have listened with great interest to Prof. VAN HOOFF's account of the valuable work carried out by him and his collaborators in the Belgian Congo. These investigations have thrown new light on a number of questions concerning the host-parasite relationships and epidemiology of sleeping sickness which have interested me for some time.

One of these is the question of reservoir hosts of the *gambian* disease. There has been a growing tendency to deny the existence of animal reservoirs of *Trypanosoma gambiense* and to regard man as the only source of infection in spite of the known fact of the survival of this trypanosome for long periods of time in antelopes. Prof. VAN HOOFF has now produced experimental and epidemiological evidence that certain domestic animals especially pigs, goats and dogs, living in close association with man in endemic areas, might serve as reservoir-hosts of human infection.

Another point of great interest brought out in this work concerns the

relationship between trypanosomes of the *brucei* group. The inhibiting effect of the blood of the host when infected with any one of the three species upon the growth in culture of all these species would seem to indicate that *T. gambiense*, *T. rhodesiense* and *T. brucei* have common antigens which give rise in the host to similar trypanocidal antibodies.

These observations lend further support to the close affinity between these trypanosomes, already established on morphological grounds.

There is one point in Prof. VAN HOOFF's communication which is not quite clear. The variations in the course of the disease observed in the Congo are attributed to the existence of strains of *T. gambiense* differing in virulence. While the role of such strains cannot be denied, it would seem that the degree of immunity acquired by the human host in certain localities might affect his susceptibility to infection and exert some influence on the course of the disease. Thus, one would expect mild or symptomless infections to occur in old endemic foci, but according to Prof. VAN HOOFF such cases are characteristic of new foci.

**Dr E. M. Lourie.** Prof. VAN HOOFF has given us a considerable number of very interesting, very important and very provocative facts and opinions, and I would like first of all to endorse Dr. CHESTERMAN's complimentary remarks about the data presented.

Prof. VAN HOOFF has naturally devoted a great deal of attention to the very striking increase in arsenic-resistance now occurring in the Belgian Congo. We are told (page 738) that the incidence of resistant cases has risen, during the past 10 years or so, from about 7 per cent to the remarkable figure of 50 or even 75 per cent in some districts. This can only be described as a very serious development, because in those areas it practically rules out the best arsenical drugs, such as tryparsamide, as major weapons in a campaign of mass-treatment for sleeping sickness. The situation seems entirely comparable with what has happened since the introduction of sulphonamide treatment for gonorrhoea. At first this was hailed as practically the end of that disease, but now we are told on all sides that gonorrhoea fails to respond adequately to sulphonamides. Here also the same question is being asked as in the case of sleeping sickness—have the strains become resistant as a result of contact with the drug in the course of years, or has the drug merely resulted in suppressing or eliminating the sensitive strains whilst allowing the naturally resistant ones to flourish? Prof. VAN HOOFF feels assured that the latter is the answer, in the case of the increasing incidence of arsenic-resistance in sleeping sickness of the Belgian Congo, and furthermore, that acquired arsenic-resistance in strains of trypanosomes tends to be lost in the course of repeated transmissions through tsetse fly. Such conclusions would, of course, be highly consoling to those who are worried by the thought that arsenic-resistant strains may have been produced actually as a direct result of their own well-intentioned (and indeed highly successful) mass-treatment programmes. But, as I believe

Prof VAN HOOFF is very willing to agree, it is extremely difficult to feel absolutely satisfied as to the interpretations placed on his experimental findings. For example, Table 8 (page 736) purports to show the establishment of a trypanosome with a lower degree of resistance at the end than at the beginning of a series of cyclical transmissions. Examination of this table seems to show however that infections of the earlier passages were generally cured by from 5 to 10 cc per kg., and that this level of curability was maintained to the end.

There is a considerable variability in the results produced by Prof. VAN HOOFF's work. Thus, resistance is sometimes acquired readily sometimes it is not. Resistance is sometimes lost easily sometimes it is not. Much of this variability is no doubt a reflection of inherent variations and instability of the trypanosomes and tsetse flies with which such work is done. One cannot escape the feeling, however that experiments carried out under field conditions are inevitably subject to many more uncontrolled and unsuspected variable factors than similar work carried out under the more precise laboratory conditions possible, for example, in this country. This is not said, in any way in disparagement of work done under field conditions. Obviously and axiomatically such work is of the very highest and first importance. There is, however more than a tendency for field workers to speak of results obtained in European laboratories as likely to be of little value because they were not obtained in the field and therefore do not apply directly to the circumstances of the field. But there does remain this fundamental advantage that, under the conditions of a well-equipped European laboratory one is able to exclude many of the variables, and so to arrive at answers which may well be ultimately of more value as pointers to what is happening in the field than are results obtained actually in the field itself. Prof VAN HOOFF's explanation of the rising incidence of arsenic resistance in the Congo may or may not be the correct one, but the fact remains that such resistance is easily produced in the laboratory and the danger was pointed out many years ago, particularly by WARRINGTON YORKE (1933), that in course of time the incidence of arsenic resistance, transmissible through tsetse fly was likely to increase in the field. It has so increased.

There is another matter on which laboratory work in this country has an important bearing. Prof VAN HOOFF tells us (page 742) that resistance to tartar emetic is now more prevalent where arsenic resistance is more common, that is, where arsenicals have been much used. Now YORKE, MURCATO and HAWKING (1932) showed years ago that it is extremely difficult, if not impossible, to produce resistance to tartar emetic when using that drug alone, but, when used in conjunction with arsenicals resistance to tartar emetic arises very readily. This appears to tie up with Prof VAN HOOFF's finding that tartar emetic resistance is particularly well known in those places where arsenicals have been much used. I have noticed, in recent accounts of work in the Congo (HAYRAUX, 1945; EDEBERT, 1946), that one of the treatments of choice for arsenic resistant sleeping sickness is tartar emetic in combination with an

## DISCUSSION

arsenical, and it appears to me that, in the light both of the old laboratory work just mentioned and of Prof VAN HOOFF's own field observations, this treatment is perhaps open to some criticism

Prof VAN HOOFF has said (page 741) that it is important to determine trypanamide-resistance in order to benefit those who carry known resistant trypanosomes by the most effective treatment against their nervous involvement. Personally, I do not know what that treatment might be. I do not know of any sound treatment for arsenic-resistant trypanosomiasis which has reached the stage of the central nervous system being involved. Prof VAN HOOFF places great faith in the new drug melarsen oxide, and it is indeed an asset that this compound should be effective against trypanamide-resistant trypanosomes. If it is effective also in the later stages of infection, then its discovery is certainly an extremely important advance, but I do not yet know of any published data to support that judgment. The original report on this drug (WEINMAN and FRANZ, 1945) does not appear to have been entirely satisfactory, and has already been criticized elsewhere (*Tropical Diseases Bulletin*, 1946). One can only assume that Prof VAN HOOFF has some data at his disposal, which he will no doubt describe in due course, in support of the faith which he places in melarsen oxide as a remedy for advanced sleeping sickness. It is, of course, always rash to prophesy, and the proof of the pudding is, after all, in the eating. Nevertheless, there are instances where one may hazard a guess, and in this case I would anticipate that the drug would not be effective against late sleeping sickness, for the reason that the strongly basic nature of its triazine nucleus would presumably militate against the likelihood of its crossing the blood-brain barrier.

Before concluding, I would like to refer to one quite different aspect Prof VAN HOOFF has spoken about the possibility of domestic animals acting as reservoirs of *Trypanosoma gambiense*. Many years ago YORKE and BLACKLOCK (1915) made a discovery, in this connection, which appears to have been somewhat neglected. They demonstrated unequivocal *T. gambiense* in an ox in Sierra Leone, and they pointed out that oxen, with other domestic animals, should accordingly be taken into account as possible reservoirs of *T. gambiense*. I notice that oxen were not mentioned by Prof VAN HOOFF, and I wonder whether they were deliberately left out, or whether Prof VAN HOOFF has some special views on the subject.

In conclusion, I must say that I am afraid most of my remarks may have sounded very adversely critical, but they were not intended to be so. I consider that the best compliment one can pay to good work is to subject it to searching criticism. Inferior work does not call for such treatment, and stands self-condemned. The excellence of Prof VAN HOOFF's contribution, representing many years of arduous labour, speaks for itself. There is, for example, his bold, and apparently highly successful, large-scale trial of mass-prophylaxis by



diamidines and antypol, and the value of Prof VAN HOOB's work as a whole cannot in any way suffer from any criticism I may have made.

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Dr VAN HOOB (in repl.) First of all I wish to convey my best thanks to Dr CHESTERMAN for the kind words he addressed to our Congo Medical Services and to myself. We do much appreciate the help that was given by the medical staff of the Missions in the struggle against all endemic diseases and especially against sleeping sickness and the Yakusu Mission brings one more example of the possibility of complete eradication of this disease by chemotherapeutic means only.

In answer to Dr DAVEY If it is true that some cryptic cases of sleeping sickness may occur after a prophylactic dose of Bayer 205 we have still no information of this happening after preventive pentamidine treatment either in volunteers or in field work.

The fact that pentamidine cannot be traced in the blood even by the most precise technique, a short time after its administration, is certainly curious in view of the long protection conferred. There remain two possible explanations. Firstly that the drug may be stored in some peculiar cells or tissues where it retains a sufficient trypanocidal activity or secondly that the drug before leaving the body may have created or increased the natural defences of the plasma.

Dr LOURIE's remarks do not seem to be in opposition to the idea of naturally resistant strains. In the case of the *Gonococcus* we have also met cases resistant to penicillin in a town where the drug had never been used before. I will not deny that in the Congo we have created, over a long period, by our chemotherapeutic work many arsenic resistant trypanosomes, but what I intended to demonstrate is that the existence of these strains is not entirely the consequence of a wrong method in our sleeping-sickness campaign.

Melarsen oxide has been tried in Léopoldville since the end of 1945 on series of recent and advanced cases and on relapse cases. Our results have not yet been published through lack of time and because many patients are still under observation and, as a rule, a definite opinion should not be given on a trypanocidal drug before sufficient time has passed to allow for eventual relapses. However what I said about the action of the melarsen oxide in nervous involvement may be taken as clearly shown in the cases we treated, and we hope to publish details very soon.

We did not mention cattle as a reservoir of trypanosomes in the endemic foci of the Congo. As a matter of fact cattle are only kept by natives outside the endemic area. Stocks owned by European enterprises do exist in the endemic areas, but almost all are in regions where only a few sporadic cases occur. These herds are not in close contact with the natives as are the smaller domestic animals. We do not ignore the danger that oxen are a possible reservoir of *T. gambiense*, for I was working in Entebbe when DUKE obtained evidence of it amongst calves at his laboratory.

Replying to Dr HOARE, I can confirm that Dr HENRARD and Mlle PEEL have worked out a series of experiments with cultures of trypanosomes which have a bearing on the difficult question of immunity and of specificity of the main groups of pathogenic trypanosomes. This work concerns also the antigenic properties of species and strains of these flagellates and will be published by the authors.

Mild cases are frequently met, usually in epidemic extensions of old foci and in new infected areas, with the characteristics of a high transmission index, although the flagellates may be very rare in the circulating blood. The majority of these trypanosomes are not resistant to tryparsamide and behave as if they were not yet altered by the influence of any drug. A small minority are resistant, and we believe that this minority of resistant strains will maintain the endemicity and even cause the gradual increase of the stock of resistant strains after a chemotherapeutic campaign.



## COMMUNICATIONS

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### INTENSIVE TRYPARSAMIDE THERAPY IN THE TREATMENT OF TRYPANOSOMIASIS

BY

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The treatment of trypanosomiasis at a general hospital in the Gold Coast presents certain difficulties. Staff specifically trained in the recognition of the disease and its treatment is not available, for the infected constitute a relatively small percentage of the total hospital attendances. The medical officer has not the time to keep accurate records and follow up cases. Hospital trypanosomiasis patients are usually treated here by twelve intravenous injections of tryparsamide at weekly intervals. In most instances the disease has progressed to the stage of advanced involvement of the central nervous system. Hence neither antrypol nor pentamidine is the drug of choice—with few exceptions. Many of the infected live in villages, often situated at a considerable distance from the hospital. An appreciable number of those who attend hospitals on main roads are migrant labourers with no fixed homes. Such cases often feel better after a few injections and then, thinking there is no need for further attendance, wander away to another locality.

Bearing in mind the above factors, especially those of distance between home and hospital and the length of time required by conservative treatment (12 weeks), it was not surprising to find during the first six months of 1943 that over half the trypanosomiasis patients attending Tamale Hospital never completed treatment. Possibly some of these became arsenic-resistant.

It occurred to the writer that an intensive course of trypanamide might circumvent many of the difficulties enumerated above. The idea was based on the short intensive method of arseno-therapy introduced by CHAGAS, LEITER and HYMAN in the treatment of early syphilis.

Seventy two consecutive cases of trypanosomiasis in varying stages of the disease were admitted to Temele Hospital in 1943 and 1944 and treated by massive dose trypanamide therapy by intravenous drip method. Later thirteen cases were treated in hospital by repeated daily intravenous injections of trypanamide in 10 c.c. double distilled sterile water using a small daily dose.

Apart from trypanosomiasis, many of these cases were also suffering from malnutrition, chronic malaria, filariasis and helminthiasis.

In all cases gland juice when available (signified by gland puncture, G.P.) thick blood films (B.L.) and cerebrospinal fluid (C.S.F.) were examined prior to treatment. Normal C.S.F. = cell count less than ten cells per c.mm. no trypanosomes, albumin 0.02 per cent. or less. C.S.F. + indicates involvement of the central nervous system (C.N.S.) as evidenced by an increased cellular content with or without an increased protein content and the presence of trypanosomes.

#### METHOD A. TREATMENT BY DRIP METHOD

(72 cases).

Total cases treated seventy two males fifty-six females sixteen.

TABLE I  
INCIDENCE AND DEATHS BY AGE GROUPS.

Age in years	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55
No. of patients	0	10	2	8		15	11	9	5	4	3
Deaths	0			0	1	2	0	1		1	2

TABLE II  
DEATHS.

G.P. + alone	BL + +ve.	C.S.F. - alone	C.S.F. - G.P. - BL -	C.S.F. - G.P. + BL +	C.S.F. - G.P. + BL +
14	0	20	70	6	2

G.P. = Gland puncture BL = Thick blood film. C.S.F. = Cerebrospinal fluid.

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The number of cases showing central nervous system involvement was fifty-six (77.7 per cent). Spinal fluid albumin was estimated by a Siccald-Cantaloube rachi-albuminometer. This was not available for the earlier cases.

### Complications

- (1) Visual disturbance, eight = 11.1 per cent
- (2) Jaundice, five = 6.9 per cent

### Mortality

Deaths in hospital eleven = 15.3 per cent (Males, eight, females, three)

### TECHNIQUE

The apparatus consisted of a douche can as container, an improvised interceptor and suitable lengths of rubber tubing. A sedative was given daily at the commencement of treatment.

The drug used was tryparsamide (May and Baker). An adult of 40 to 50 kg in weight was given about 2 grammes in 2 pints double distilled sterile water daily for 6 to 9 days. It was often found necessary to stop treatment for a day or two during this course as the high temperature resultant upon treatment was exhausting.

All the drip apparatus was sterilized and then flushed through with sterile distilled water. The patient's arm was immobilized on a splint. The container having been filled, a vein on the forearm was entered and connected with the apparatus, which was already dripping to exclude air bubbles. A rate of about forty drops a minute allowed 2 pints of solution to flow in about 8 hours.

The three cases, Cases 34, 4, 51, which showed no improvement as judged by laboratory findings, are of interest. Case 4 denied ever having had previous treatment for the disease but may well have been infected with an arsenic-resistant strain of trypanosome. Cases 34 and 51 were both treated at an earlier date at Tamale Hospital with tryparsamide. Apart from that fact, no information could be obtained about these two cases from hospital records. It seems probable that both had been rendered arsenic-resistant by their previous treatments.

Alternate arms were used each day.

### Observations During Treatment

- 1 Pyrexia, often very high, was recorded in every case under treatment. It is impossible to produce pyrogen-free water with the type of still in use in this colony. This pyrexia may enhance the action of the tryparsamide (cf pyro-therapy in the treatment of syphilis) but it is exhausting and dangerous.
- 2 Pain along the course of the vein was common, but constituted only a temporary inconvenience.

TABLE III  
THIRTY NINE CASES FOLLOWED UP

	Case Number	Stage	Result
Group 1 (eight cases). 2-6 months follow up	26	Early	Improved.
	22		Satisfactory
	41	C.N.S. involved	Improved.
	3		
	54		
	64		
	31		
	31		Satisfactory No improvement. Died March, 1945. Probably arsenic resistant.
Group 2 (one case) 6-12 months follow up	14	Early	Satisfactory
Group 3 (ten cases). 13-18 months follow up	47	Early	Satisfactory
	56		
	59	C.N.S. involved	
	13		
	60		
	63		
	63		
	45		
	4		} No improvement. Probably arsenic-resistant.
	81		
Group 4 (re-entry cases) 18-4 months follow up.	2	Early	Satisfactory
	6		
	7		
	16		
	20		
	25		
	28		
	40		
	8		
	15		
	16		
	17		
	19		
	21		
	23		
	24		
	25		
	26		
	29		
	39		

Early = no involvement of central nervous system (C.N.S.).

Trypanosomiasis being a disease of considerable chronicity no claim is made of cure of the above cases. The word satisfactory is used to indicate negative blood and gland juice (G.P.) (if glandular enlargement present) and normal spinal fluid, when the case was last seen.

3 The treatment caused much less disturbance and weakness in children than in adults

4 Five cases showed a trace of bile in the urine at the completion of treatment This was not associated with any other evidence of liver damage and cleared rapidly when treatment ceased It is of interest to note that bile in the urine only occurred when other cases of liver disease were present in the wards

5 In eight cases optic neuritis occurred during treatment With one exception these were cases with advanced involvement of the central nervous system The degree of visual disturbance varied from slight blurring of vision to perception of light only In all instances the onset was sudden Treatment was stopped and intravenous sodium thiosulphate given daily for 4 to 5 days Two patients were not benefited by this and their vision remained very poor One of these subsequently died (Case 60) In five cases visual acuity improved considerably and the final impairment was not sufficient to interfere with resumption of normal occupation, e g, farming

6 There were eleven deaths during treatment In most of these cases the general condition had been described as "poor"

#### RESULTS OF TREATMENT

(a) *Immediate*—Clinically, most patients appeared to be considerably benefited There was a rapid subsidence of the enlarged post-cervical glands After treatment, those glands, which were still large enough to puncture, gave a negative result The rapid loss of somnolence and improvement in mental acuity was frequently dramatic

In assessing the amelioration of symptoms, it must be remembered that tryparsamide has a tonic effect, that Gold Coast Africans consider any form of treatment by injection highly beneficial and therefore will say they are better after any injection, that the type of patient under consideration will complain only of the grosser symptoms

(b) *Late Results*—Those cases which could subsequently be traced can be classified into four groups (Table III, page 766)

Group	1	Those examined	2 to	6 months after treatment
"	2	"	"	6 " 12 " " "
"	3	"	"	12 " 18 " " "
"	4	"	"	18 " 24 " " "

A summary of Table III shows that of the thirty-nine cases followed up there was no change, in 3, improved, 4, apparently cured, 32



## CONCLUSIONS.

Although the results of this drip method of treatment were frequently good and the length of treatment considerably curtailed, the mortality rate was high (15.3 per cent.) and the incidence of visual disturbance also high (11.1 per cent.).

Apart from the selective action of trypanamide on the optic nerve, no other toxic effects were seen. It is considered that the slight jaundice seen in a few cases was not due to the drug.

The deaths with one exception, were probably due to the exhausting effect of a high temperature on a tired myocardium.

Unless therefore, pyrogen-free water is available this method of treatment is not recommended. Hospitalization is essential and it is time absorbing to the nursing staff who are always in short supply. Also it may well be that Method B (smaller daily injections) will give good results and have practical application in the treatment of trypanosomiasis both in hospital and in the field.

The importance of examination of the spinal fluid in diagnosis and subsequently is stressed. Likewise all cases presenting rheumatic symptoms should be examined to exclude trypanosomiasis. A facile diagnosis of "yaws rheumatism" will result in many cases of trypanosomiasis being missed.

#### METHOD B. TREATMENT BY DAILY 10 C.C. INTRAVENOUS TRYPARAMIDE INJECTION (THIRTEEN CASES)

As stated earlier it is impossible to produce pyrogen free water at Tsimale Hospital. The constitutional disturbance resultant upon the use of water containing pyrogens in the drip method was probably responsible for the high mortality rate. It was therefore decided to give the trypanamide dissolved in 10 c.c. distilled water daily in smaller doses over a rather longer period of time.

This method has the merit of simplicity. The setting up of an intravenous drip apparatus to maintenance for 8 hours daily and the resultant pyrexia, necessitated a considerable amount of additional work for the staff. The single daily 10 c.c. injection has none of these objections and could be used for out patients.

Total cases treated thirteen. (males nine females, four)

TABLE IV  
AGE GROUP INCIDENCE.

Age in years	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50
Number of patients	1	1	1			1		4	1	1

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TABLE V  
DIAGNOSIS

GP + alone	Bl + alone	CSF + alone	CSF + GP + Bl —	CSF + GP + Bl +	CSF — GP + Bl +
0	0	6	7	0	0

Number of cases showing CNS involvement 13 (= 100 per cent)

Number of cases showing CNS involvement, thirteen = 100 per cent

### Observations During Treatment

No toxic effects were seen in these cases during treatment with the exception of visual disturbance—optic neuritis—in Case 13. There were no deaths.

All these cases showed evidence of CNS involvement and in most instances the involvement was advanced.

### Results

At the conclusion of treatment, there was considerable clinical improvement in all cases, but when using tryparsamide, clinical improvement is not necessarily commensurate with improvement in the laboratory findings.

Only three cases, Cases 2, 6, 11, could subsequently be followed up.

### CONCLUSIONS

The total quantity of tryparsamide given was variable, as the tryparsamide requirement was not known. Cases 2, 6, 11, which were seen about 10 months later and appeared to be cured, were given 18, 14½, 16 grammes each respectively. It is suggested that 14 to 16 grammes tryparsamide would probably be adequate for an adult of about 50 kg, who showed moderately advanced CNS involvement.

These cases were not lumbar punctured immediately after treatment, as the results of examination of spinal fluid immediately after treatment can be very misleading. Not infrequently the cell count has risen considerably. A more reliable record is obtained if the spinal fluid is examined a month or two after treatment.

The number of cases treated is too small to provide material from which conclusions of value can be drawn. It is, however, suggested that further cases should be treated by this method and followed up, in view of the good late results in Cases 2, 6 and 11.

## SUMMARY

Treatment of seventy two cases of trypanosomiasis by intensive try parsamide drip therapy is described. Late results appear to be satisfactory but the method is not recommended under existing conditions owing to the high mortality rate.

Treatment of thirteen cases similarly affected by daily intravenous try parsamide in 10 c.c. solution is also described. This method requires further investigation and may be of considerable value.



3 To study the influence exerted by the detoxication of venoms on the same hyperglycaemic action, particularly with a view to elucidating whether the hyperglycaemic action is linked up with the toxic properties of venoms or eventually with their antigenic properties. Such experiments are easily carried out by using detoxicated venoms (anavenoms) which allow high concentrations of venom derivatives to be injected in atoxic form. (GRASSET and ZOUTENDYK, 1932 RAMON 1925)

In contrast to BERTRAND and VLADESCO who employed guineapigs mostly for their experiments we used rabbits exclusively. Intravenous injections into rabbits are technically easier than into guineapigs especially when large volumes are injected. Also successive venous bleedings can be carried out with ease on rabbits without having to resort to cardiac punctures as in the case of guineapigs. As mentioned by BERTRAND these punctures may be accompanied by variations in the blood sugar content even without the administration of venom, when guineapigs are used.

#### DETERMINATION OF BLOOD SUGAR IN NORMAL RABBITS.

Before proceeding to study the eventual hyperglycaemic action of venom on rabbits, we did a series of blood sugar determinations on normal rabbits submitted to three successive bleedings to ascertain the extreme normal limits under similar experimental

TABLE I

NORMAL RABBITS—BLOOD SUGAR CONTENT IN MG. PER 100 C.C.

A—Starved Rabbits.

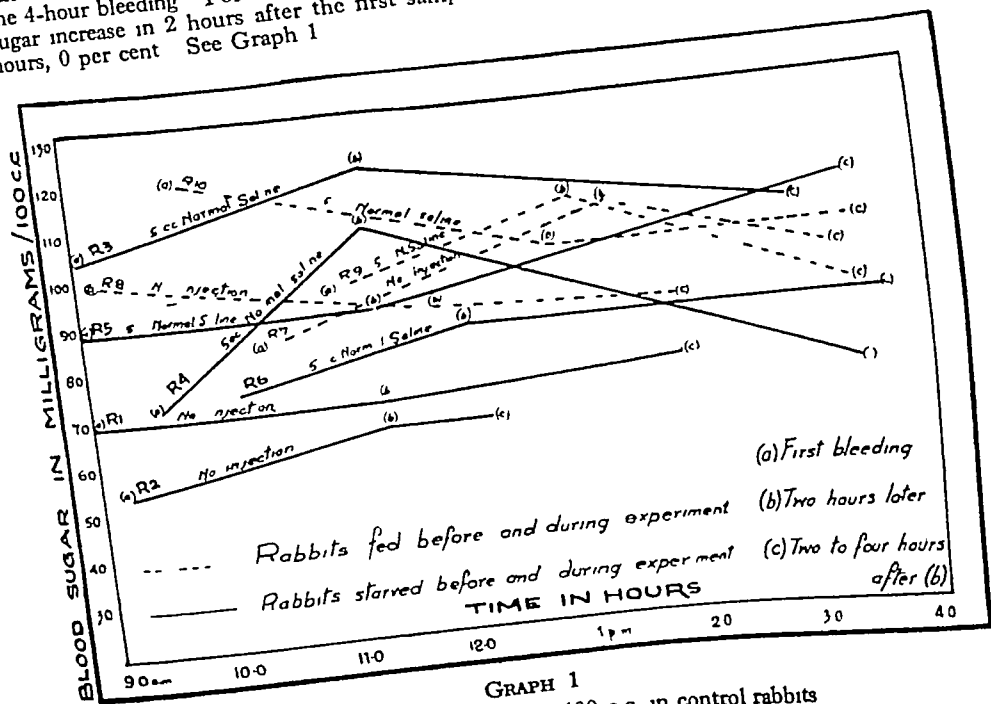
Number	Weight in grammes.	Injection	First bleeding before injection.	Second bleeding 2 hours later	Third bleeding 3 to 4 hours later	Per cent. variation in blood sugar at second bleeding.	Per cent. variation in blood sugar at third bleeding.
1	1,978	None	78	76	73	0	+ 7.1
2	2,270		85	85	85	+19.1	+18.1
3	2,300	S. c. saline	100	120	103	+14.27	0
4	2,600		70	105	70	+50.0	0
5	2,090		80	90	110	0	+22.2
6	2,800		75	85	84	+12.2	+12.2
B—Fed Rabbits.							
7	2,430	None	85	105	84	+22.9	0
8	2,250		100	90	85	-10	-15
9	2,250	S. c. saline	85	110	95	+15.0	0
10	1,850		120	108	100	-14.4	-16.6

conditions. No reference could be traced in the literature to blood sugar estimation in rabbits. In order to eliminate as much as possible variations due to feeding, some rabbits used in these tests were started during the previous night from 6 p.m. and during the whole time of the experiment, i.e., the following day. They were supplied, however, with drinking water during this period. The weight of the rabbits varied from 1,600 to 3,400 grammes. The method used throughout our experiments for determining the blood sugar content was that of MACLEAN (1924).

These starved rabbits were submitted the following morning to three successive bleedings of 2 to 3 c.c. of blood taken from the lateral ear vein. The first sample was usually taken between 9 and 10 a.m. The second sample was collected 2 to 3 hours later, between 11 and 12 a.m., and the third, 2 to 3 hours later again, i.e., 4 to 6 hours after the original bleeding.

The blood was rendered non-coagulable by the addition of 0.5 per cent sodium citrate solution in the collection tube. All blood sugar determinations were carried out within the hours immediately following bleeding. For each blood sample examined, two to three independent tests were carried out and the average taken as the final figure. These figures are expressed in mg. of glucose per c.c. of blood. Table I (A) shows the results obtained in a series of six normal starved rabbits. In Rabbits 3, 4, 5 and 6, 5 c.c. of saline were injected intravenously after the first sample bleeding, being the same volume used for venom injections in subsequent experiments. Figures for Rabbits 7 to 10 are those for rabbits which were fed before and during the experiment.

For the starved rabbits, Rabbits 1 to 6, an average blood sugar increase of 15.9 per cent was observed in the 2-hour sample bleeding and an increase of 12.6 per cent in the 4-hour bleeding. For the rabbits which were fed, Rabbits 7 to 10, the average blood sugar increase in 2 hours after the first sample bleeding was 9.82 per cent and after 4 hours, 0 per cent. See Graph 1.



GRAPH 1  
Blood sugar in milligrams per 100 c.c. in control rabbits

### HYPERGLYCAEMIA IN RABBITS SUBMITTED TO INJECTIONS OF *Naja flava* VENOM.

The venom used in these experiments was a pooled mixture of 20 grammes of *N. flava* venom, collected in Cape Province in 1942, from more than 100 specimens of Cape cobra, and kept since that time in a pulverized form in closed containers. The lethal dose of this venom for rabbits of 2,000 to 2,200 grammes weight is 0.35 mg. by intravenous injection and 1.4 mg. by subcutaneous injection. Death occurs in 4 to 6 hours.

#### DETERMINATION OF BLOOD SUGAR CONTENT FOLLOWING SUBCUTANEOUS INJECTION OF *Naja flava* VENOM.

In this experiment, starved rabbits were bled (1) immediately before administration of the venom (2) 2 hours after the injection of 1.4 mg. of *N. flava* venom administered *subcutaneously* in the flank (3) the final bleeding was done within a period varying from 4 to 8 hours after the venom injection and soon before death, when the animals were showing very advanced signs of intoxication such as paralysis and dyspnoea. The blood sugar content for six rabbits so treated is given in Table II.

TABLE II.  
BLOOD SUGAR CONTENT IN mg. PER 100 G.

Start of Rabbits.

Number	Weight in grammes.	<i>N. flava</i> venom injected subcutaneously	First bleeding before injection	Second bleeding hours later	Third bleeding 3 to 4 hours later	Per cent. variation in blood sugar at second bleeding	Per cent. variation in blood sugar at third bleeding
11	2,630	1.4 mg.	90	90	163	+12.3	+31.3
12	2,733	1.4	70	70	193	0	+178
13	2,409	1.4	88	83	183	+41.6	+128
14	2,370	1.4	63	80	99	+23.0	+39.4
15	2,375	1.4	70	—	313	—	+441.1

The average percentage increase in blood sugar 2 hours after the venom injection was 19.2 per cent. and the increase before death (1 hour 40 minutes to 6 hours after the injection) was 145 per cent. It is important to note the individual differences in the rise of blood sugar following the same dose of venom: the increase varied from 31.3 to 441.1 per cent. In two of the animals (Rabbits 11 and 14) the increase took the form of a moderate continual rise while in the others the initial moderate increase was followed by a sharp rise during the hours prior to death.

As a point of interest we here describe a similar experiment carried out under analogous conditions except that the rabbits under test were *not starved* before the injection of venom. The results obtained are incorporated in Table III.

TABLE III  
BLOOD SUGAR CONTENT IN MG PER 100 C.C.

*Fed Rabbits*

Number	Weight in grammes	<i>N. flava</i> venom injected subcutaneously	First bleeding before injection	Second bleeding 2 hours later	Third bleeding 3 to 4 hours later	Per cent variation in blood sugar at second bleeding	Per cent variation in blood sugar at third bleeding
16	2,260	1.4 mg	150	130	295	-13.3	+96.7
17	2,650	1.4 "	180	120	Died after second bleeding	-33.3	—
18	2,185	1.4 "	85	—	120	—	+41.2
19	1,850	1.4 "	90	115	150	+27.8	+66.7
20	1,770	1.4 "	80	63	100	-21.3	+25.0

It will be seen that in the fed rabbits the average percentage increase in blood sugar, 2 hours after the venom injection, was +6.95 per cent, and the increase soon before death (2 to 4 hours after the injection) was +57.4 per cent. Two points are noteworthy: (1) The higher original sugar content in the fed rabbits as compared with that in the starved specimens, (2) the initial drop observed in three out of the five fed rabbits at the second bleeding. This drop was respectively -21.3, -13.3 and -33.3 per cent—a contrast to the moderate, initial increase observed in the starved rabbits. This initial drop in the fed rabbits is followed by a blood sugar increase of relatively smaller magnitude (average increase 57.4 per cent) when compared with the increase in the starved rabbits (average increase 145 per cent).

#### DETERMINATION OF BLOOD SUGAR CONTENT IN RABBITS SUBMITTED TO INTRAVENOUS INJECTIONS OF *Naja flava* VENOM

Eight rabbits, starved as before, were submitted to *intravenous* injections of *N. flava* venom.

Four rabbits were injected with 0.35 mg. of *N. flava* venom in 5 c.c. saline, this being the dose of venom necessary to kill rabbits of 2,200 to 2,800 grammes in 1 to 4 hours. A sample of blood was taken just before the injection of venom. The second bleeding was made after a somewhat shorter interval than in the case of the subcutaneous injections (i.e. 1 to 1 hour 30 minutes). The



third bleeding was taken soon before death, i.e., 2 to 4 hours after the administration of venom. The blood sugar estimations recorded are given in Table IV

TABLE IV

BLOOD SUGAR CONTENT IN MG. PER 100 C.C.

Starved Rabbits.

Number	Weight in grammes.	<i>N. fies</i> venom injected intravenously	First bleeding before injection.	Second bleeding 2 hours later	Third bleeding 3 to 4 hours later	Per cent. variation in blood sugar at second bleeding	Per cent. variation in blood sugar at third bleeding.
21	~800	0.33 mg.	83	90	70	+ 33.4	+ 7.6
22	~830	0.33	70	140	170	+100	+142.4
23	2,710	0.33	85	160	180	+ 17.6	+111.4
4	2,370	0.25	25	—	312	—	+782.4

Again all rabbits injected showed a blood sugar increase which in this experiment averaged 52 per cent. after 1 hour 30 minutes and 263 per cent. after the third bleeding soon before death, reaching in the case of Rabbit 24, an increase of 782.4 per cent. The increase of blood sugar appears to be more accentuated when death takes place only after an hour. Results observed in Rabbit 21 are of particular interest from this point of view. Rapid evolution of the symptoms was followed by early death in little over an hour. The rise in the blood sugar content observed during the 1st hour was followed by a rapid decrease almost approaching the initial level.

In the next experiment, four other rabbits were given a slightly lower dose (0.3 mg.) of *N. fies* venom intravenously. This amount of venom is sufficient to provoke severe symptoms of venom intoxication in rabbits, resulting in delayed death with an occasional recovery. Of these four rabbits, three (Rabbits 25, 27 and 28) died after 4 to 6½ hours the other was bled in *extremis* after 27 hours (Table V, page 778).

In these rabbits a marked increase in blood sugar was observed within the hours immediately following the administration of venom—the average increase at the second bleeding was 66.4 per cent. The average increase at the third bleeding was 116.8 per cent. The *highest maximum* increase was reached just before death in the three rabbits which died within 6 hours. In the fourth rabbit, Rabbit 26 the increase after 4 hours was 114.2 per cent., but after 24 hours there was a fall to — 33 per cent. below the original blood sugar level. Graph 2 shows the successive blood sugar levels for the respective rabbits with the corresponding times at which the bleedings were taken and the periods after which death ensued.

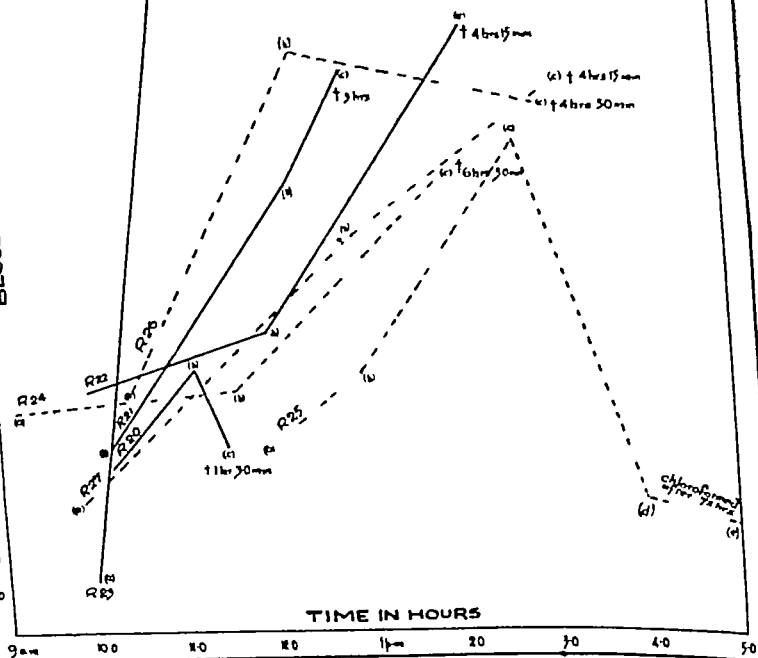
GRAPH No 2

GRAPH TO SHOW  
BLOOD SUGAR IN MILLIGRAMS PER 100cc  
IN RABBITS INJECTED INTRAVENOUSLY  
WITH NAIA FLAVA VENOM

----- Rabbits injected with 0.1 mgm of Naia Flava venom in 5 ccs Normal Saline  
—— Rabbits injected with 0.35 mgm Naia Flava venom in 5 ccs Normal Saline

- (a) Before injection  
(b) Two hours later  
(c) Before death  
† Death

BLOOD SUGAR IN MILLIGRAMS / 100cc



From the above experiments it appears that injection of *N. ferox* venom administered in adequate doses either subcutaneously or intravenously to produce death within a few hours, is followed by a considerable increase in the blood sugar content of the animals so treated. The intravenous injection of *N. ferox* venom causes a much sharper initial rise than the subcutaneous administration of larger doses of the same venom. The lesser reaction of the latter appears to be related to the progressive absorption of venom but after the initial phase there is a rapid hyperglycaemic rise, as with intravenous injections, which reaches in some starved rabbits an increase of 178 and 411 per cent. over the original blood sugar level.

Rabbits fed prior to the subcutaneous injection show on the whole a higher original blood sugar level. A slight fall in the blood sugar level is observed during the 2 hours following the venom injection, followed by a sharp hyperglycaemic rise. This initial negative phase contrasts with the continual rise in hyperglycaemia which appears to start soon after the injection of venom in starved rabbits.

TABLE V  
BLOOD SUGAR CONTENT IN MG. PER 100 C.C.

Starved Rabbits

Number	Weight in grammes	<i>N. ferox</i> venom injected intravenously	First bleeding before injection	Second bleeding 2 hours later	Third bleeding 3 to 4 hours later	Per cent. variation in blood sugar at second bleeding	Per cent. variation in blood sugar at third bleeding
5	2,300	0.3 mg	60	85	140	+ 6.5	+ 75
26	275	0.3	75	90	180	+ 28.8	+114.3
27	2,450	0.3	85	175	160	+108.8	+ 86
28	2,175	0.3	85	125	180	+157.6	+180

Delayed deaths appear in some cases to be accompanied by a secondary drop from the hyperglycaemic peak reached, down to hypoglycaemic levels below the level existing before the injection of the venom. The hyperglycaemic levels obtained with *N. ferox* venom are, on the whole, of the same magnitude as those obtained by BERTRAND and VLADESCO (1940) with colubrine venoms. These authors injected only two guinea-pigs with *N. ferox* venom. A 100 per cent. blood sugar increase was observed in one guinea-pig after a subcutaneous injection of 13 mg with death following in 6 hours. In the second guinea-pig, which received 2 mg in two fractions at hourly intervals, the rise was limited to 38 per cent. and the guinea-pig survived. They also quoted a 155 per cent. blood sugar increase for a guinea-pig injected with *N. Aspis* venom. The

maximum increase they recorded for colubrine venom was 175 per cent in a guineapig injected subcutaneously with 2.35 mg of *Dendropsis angusticeps*

### HYPERGLYCAEMIA IN RABBITS SUBMITTED TO INTRAVENOUS INJECTIONS OF *Bitis arietans* VENOM

The venom of South African viperines used in these experiments was part of a pooled, pulverized mixture of about 6 grammes collected in 1939 from over thirty specimens of *Bitis arietans*. Its toxicity was periodically checked in antivenene standardization work. The intravenous injection of 1.2 mg of this venom to rabbits of 2,000 to 2,200 grammes produced marked symptoms of intoxication followed in most cases by recovery but occasionally by late death after 10 to 16 hours. A dose of 1.4 mg caused, soon after injection, symptoms of progressive severity, usually terminated by death in 3 to 4 hours. While with *N. flava* venom, death is mainly due to the neurotoxic principle characterizing colubrine venoms, with *B. arietans* proteolytic and haemorrhagic disorders play an important part in the pathology of the intoxication.

In the first experiment with *B. arietans*, two starved rabbits received a sublethal dose of 1.2 mg of venom given intravenously with saline in a volume of 4 c.c. As with the *N. flava* experiments, a first sample of blood was taken immediately before venom was injected, a second 2 hours later when the animals were manifesting definite signs of intoxication, and a third 3 to 4 hours later when the condition of the rabbits was apparently stationary or showing a tendency towards recovery.

In Table VI, Rabbits 29 and 30 given a sublethal dose of venom, showed only a slight or moderate increase in blood sugar content. After a short rise, following the injection of venom the blood sugar level dropped towards the

TABLE VI  
BLOOD SUGAR CONTENT IN MG PER 100 C.C.

Number	Weight in grammes	<i>B. arietans</i> venom injected intravenously (in mg)	First bleeding before injection	Second bleeding 2 hours later	Third bleeding 2 to 4 hours later	Interval from venom injection to death (in hours or minutes)	Per cent variation in blood sugar at second bleeding	Per cent variation in blood sugar at third bleeding
29	2,400	1.2	55	80	65	No death	+ 45.5	+ 18.1
30	1,875	1.2	90	130	125	"	+ 44.0	+ 39.0
31	1,900	1.3	55	—	30	3 hrs 35 mins	—	— 45.5
32	2,380	1.4	65	250	275	3 " 40 "	+ 284.6	+ 323.0
33	2,450	1.4	75	215	300	2 " 15 "	+ 186.7	+ 300.0
34	2,350	1.4	65	—	313	2 " 15 "	—	+ 381.6

normal level while the intoxication symptoms subsided. Rabbit 31 is recorded as an exceptional case—the injection of Rabbit 31 with a slightly higher dose, 1.3 mg of *B. aristatus* venom, resulted in early death within 4 hours. This rapid evolution of symptoms was accompanied by a lowering in the blood sugar content before death. We cannot give any explanation for the cause of this unusual observation. It is possible that sudden death followed on thrombosis and embolus and possibly was not due to general intoxication.

In a further experiment, three rabbits were injected intravenously with 1.4 mg *B. aristatus*, i.e., the certainly lethal dose of venom (C.L.D.) for rabbits of 2,000 grammes. Blood sugar determinations were carried out as previously before venom administration, 2 hours afterwards, and soon before death (in this case 2 to 4 hours from the time of the venom injection).

The results of these experiments are incorporated in Table VI (Rabbits 32-34).

The blood sugar changes were of the same character as those which occurred in the *N. ferox* experiments. When *B. aristatus* was administered in fatal doses by the intravenous route, the blood sugar content showed a rapid rise within 2 hours of the injection. This sharp hyperglycaemic rise is maintained during the following hours, reaching its maximum soon before death, the increase varying from 300 to 381.6 per cent. Details of these experiments are shown in Graph 3. The hyperglycaemic levels reached with *B. aristatus* are on the whole higher than those obtained with *N. ferox* venom.

A second experiment was carried out with another specimen of *B. aristatus* venom of lower toxicity. 1.4 mg injected intravenously in rabbits resulted only in late death—after 12 to 31 hours. A higher dose of 1.8 mg proved fatal in 1½ to 5 hours.

Starved rabbits of 2,000 to 2,600 grammes were injected respectively with doses of 1.4, 1.6 and 1.8 mg of this venom, and blood sugar determinations were carried out on a similar basis as in the previous experiments. As shown by the results of this experiment in Table VII, the lower doses of 1.4 and 1.6 mg. of venom produced an increase in blood sugar after 2 hours. These were followed, however, by a subsequent drop towards the original blood sugar level during the following 12 to 24 hours preceding death.

With the higher dose of 1.8 mg. venom, the early 2-hour blood sugar increase was maintained and found to be somewhat higher soon before death (Rabbits 40 and 41). Details of these experiments are shown in Table VII.

Reference to only one guinea-pig injected with *B. aristatus* is given in BERTRAND's second publication (BERTRAND and VLADESCO 1940a). This animal received 4.5 mg of *B. aristatus* subcutaneously split into two doses of 2.6 and 1.9 mg at ½ hour intervals. Blood sugar determinations before venom injection and 9 hours later at time of death, are reported as 0.735 and 0.13 mg. respectively representing a hyperglycaemic rise of 75 per cent.

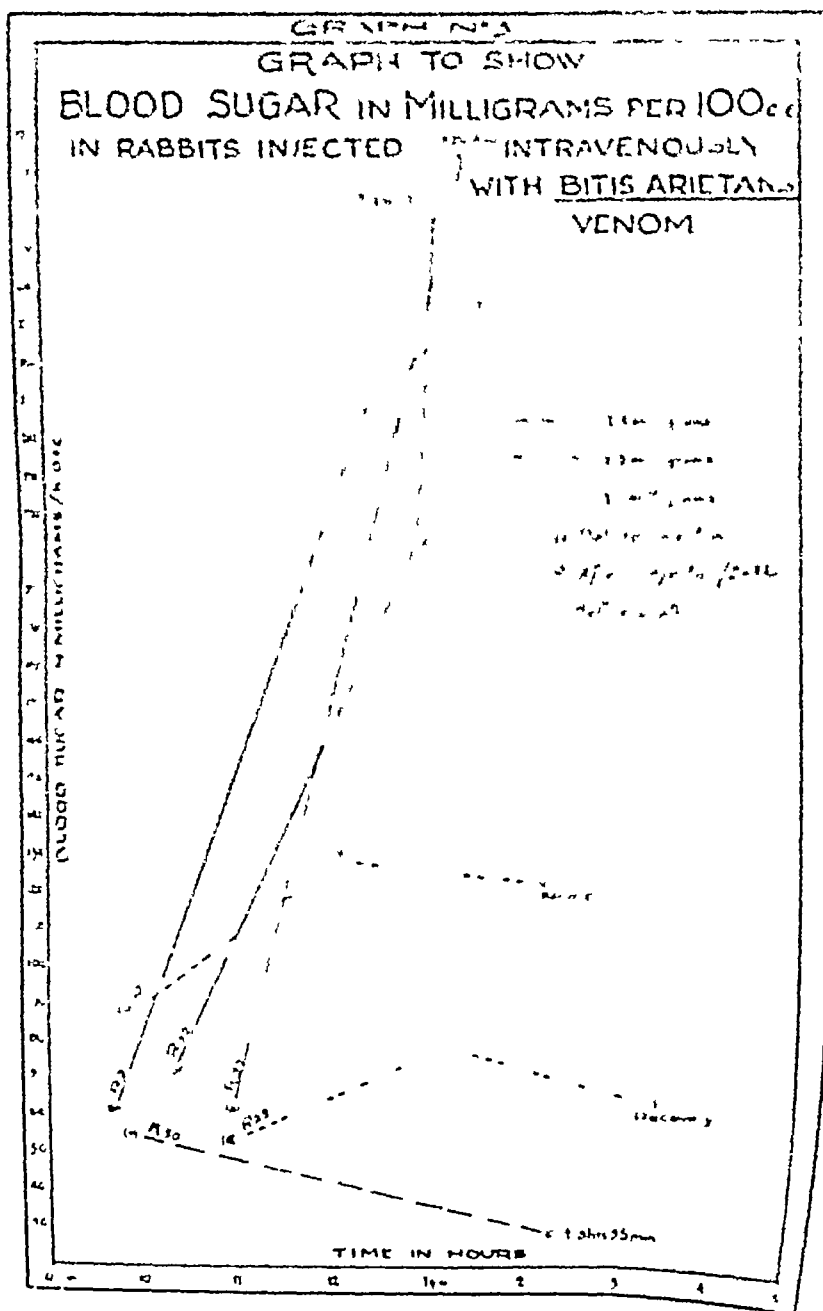


TABLE VII.

BLOOD SUGAR CONTENT IN MG. PER 100 C.C.

Starved Rabbits.

Num- ber	Weight in grammes.	<i>B. asietanus</i> venom injected intrave- nously (in mg.).	First bleeding before injection.	Second bleeding 2 hours later	Third bleeding 3 to 4 hours later	Hours from venom injection to death.	Per cent. variation in blood sugar at second bleeding.	Per cent. variation in blood sugar at third bleeding.
24	2,550	1.4	70	140	70	31	+100	0
36	2,270	1.4	55	120	75	12	+118.1	+96.3
37	~000	1.4	70	120	105	12	+71.4	+99
38	2,400	1.4	80	140	70	4½	+75.0	-12.5
39	~050	1.8	75	—	30	2½	—	-80
40	2,000	1.8	70	100	200	1½	+171.4	+183.7
41	1,180	1.8	75	115	1.5	5½	+83.3	+98.4

Furthermore the considerably higher hyperglycaemic action observed in our experiments (two to four times higher) may partly be attributed to the more rapid and intense action of venom injected intravenously without splitting the amount injected into two doses.

#### NEUTRALIZING INFLUENCE OF SPECIFIC ANTIVENOMOUS SERUM ON VENOM WITH REGARD TO THE HYPERGLYCAEMIA EXERTED BY THE VENOM.

The following experiments were carried out with a view to ascertaining whether the hyperglycaemic action exerted by venoms would be influenced when venoms are neutralized by specific antivenene prior to injection. Besides its academic interest it was estimated that this particular approach to the subject might be of practical utility in the treatment of snakebite.

#### DETERMINATION OF BLOOD SUGAR CONTENT IN RABBITS SUBMITTED TO INJECTIONS OF NEUTRALIZED *Naja flavo* VENOM-SPECIFIC ANTIVENENE MIXTURE.

The same sample of *N. flavo* venom was used as in the previous experiment. Two mg. of this venom was mixed with 3 c.c. of concentrated anti-*N. flavo* serum. These proportions ensure full venom neutralization. 125 c.c. of this specific serum is sufficient to neutralize *in vitro* 1 mg. of *N. flavo* venom.

After 1 hour contact at 37° C. this mixture was injected intravenously in each of three starved rabbits in the volume of 5 c.c. made up with saline. Blood sugar determinations were carried out as previously on three sample bleedings, i.e. before injection of the mixture 2 hours and 4 hours later. The

rabbits so treated remained normal throughout the experiment. The results of the corresponding blood sugar tests are recorded in Table VIII.

A slight blood sugar increase from 28.5 per cent to 36 per cent was observed during the 4 hours following the injection of venom-antivenene mixture.

Another rabbit was injected with a mixture containing the same venom-antivenene proportions as above but in double the volume, i.e., 4 mg of *N flava* venom added to 6 c.c. of specific serum. The corresponding blood sugar determinations (Table VIII, Rabbit 47) show a similar moderate rise as in the previous experiment despite the double quantity of venom in the last mixture.

The results contrast with the considerably higher hyperglycaemic rates observed (three to five times higher) when *N flava* venom was injected in the absence of serum and in amounts ten to thirteen times lower (0.3 to 0.35 mg.)

TABLE VIII

BLOOD SUGAR CONTENT IN MG PER 100 C.C.

Starved Rabbits

Number	Weight in grammes	<i>N flava</i> antivenene mixture	First bleeding before injection	Second bleeding 2 hours later	Third bleeding 3 to 4 hours later	Per cent variation in blood sugar at second bleeding	Per cent variation in blood sugar at third bleeding
42	1,975	2 mg V + 3 c.c. S	70	80	95	+ 14.3	+ 36
43	1,650	2 " V + 3 " S	90	110	120	+ 22.2	+ 33.3
44	2,430	2 " V + 3 " S	70	70	90	0	+ 28.6
45	2,180	1.6 " V + 4 " S	120	100	100	- 16.7	- 16.7
46	2,080	3.2 " V + 8 " S	35	110	85	+ 214	+ 142.8
47	2,000	4 " V + 6 " S	70	90	90	+ 28.3	+ 28.3

V = Venom      S = Serum

As a matter of interest, the following experiment, although difficult in its interpretation, is given —

Two rabbits were injected with *N flava* venom-antivenene mixtures, containing a large excess of antitoxin: (1) 1.6 mg *N flava* venom + 4 c.c. antivenene, and (2) the same mixture but doubled in volume, i.e., 3.2 mg venom + 8 c.c. antivenene. As shown in Table VIII, hypoglycaemia (— 16.7 per cent) was noted in Rabbit 45, injected with mixture (1), whilst a considerable increase was observed in Rabbit 46, injected with double volume of the same mixture (2). This exceptional and unexpected blood sugar increase observed with a venom content and ratio lower than in previous experiments, may be connected with the relatively large amount of foreign serum (8 c.c.) introduced intravenously.



DETERMINATION OF BLOOD SUGAR IN RABBITS SUBMITTED TO INJECTIONS OF NEUTRALIZED *B. arisae* VENOM SPECIFIC ANTIVENENE MIXTURE.

The same type of experiment was repeated for *B. arisae* venom-antivenene mixture.

The specific anti *B. arisae* serum used neutralized 10 mg. of *B. arisae* venom per c.c. i.e., a neutralization rate about ten times higher than in the case of *N. ferox* venom. A mixture containing 1.5 c.c. of antivenene and 10 mg. *B. arisae* venom was injected intravenously after 1 hour contact at 38° C. to each of three starved rabbits. These proportions ensure full venom neutralization. Three other starved rabbits were injected with a double amount of the same mixture, i.e. representing 20 mg. of venom + 3 c.c. antivenenous serum. All injections were administered intravenously in a total of 10 c.c. complete with saline.

Blood samples were taken as previously i.e., before injection, 2 hours and 4 hours later. The results of the determination of blood sugar content for the respective animals are given in Table IX.

TABLE IX.  
BLOOD SUGAR CONTENT IN MG. PER 100 C.C.

Starved Rabbits.

Number	Weight in grammes.	Venom antivenene serum mixture.	First bleeding before injection	Second bleeding 2 hours later	Third bleeding 2 to 4 hours later	Per cent. variation in blood sugar at second bleeding	Per cent. variation in blood sugar at third bleeding
48	2,200	10 mg. V + 1.5 c.c. S	68	78	83	+15.4	+18.4
49	2,225	10 V + 1.5 S	103	95	95	- 9.8	- 9.5
50	2,970	10 V + 1.5 S	90	76	93	- 6.25	- 0.25
51	2,850	20 V + 3 S	79	100	100	+42.6	+42.8
52	2,900	20 V + 3 S	78	75	79	0	- 0.5
53	2,370	20 V + 3 S	70	90	70	+14.2	0

V = Venom. S = Serum.

Average blood sugar increase for 10 mg. V + 1.5 c.c.S. In second bleeding + 5.13 per cent. in third bleeding 0 per cent. Average blood sugar increase for 20 mg. V + 3 c.c.S. In second bleeding + 14.3 per cent. in third bleeding + 14.3 per cent.

As in the case of the *N. ferox* venom-antivenene neutralized mixture only a slight or moderate increase in blood sugar was observed during the 4 hours following its injection. Except in one case, Rabbit 51 this was soon followed by a drop to original normal levels.

It is noteworthy that for Rabbits 51, 52 and 53, the amount of *B arietans* venom contained in the mixture injected, *i.e.*, 20 mg, was fourteen times higher than the amount found sufficient (1.4 mg) to produce a 400 per cent blood sugar increase when injected to rabbits in the absence of specific serum.

It appears, therefore, that the hyperglycaemic action exerted is related to the toxic form under which venoms are introduced to the system, and not to the actual amount of venomous proteins injected. Once venom has been neutralized by specific antivenene, its actual amount in the admixture has no direct effect on the phenomenon.

#### DETERMINATION OF BLOOD SUGAR IN RABBITS SUBMITTED TO INJECTIONS OF DETOXICATED VENOMS (ANAVENOMS)

The following experiments were carried out to ascertain whether the hyperglycaemic action of venoms would be influenced after the latter had been detoxicated. Such investigations could be realized by means of atoxic venoms, "anavenoms", resulting from the treatment of venoms with appropriate amounts of formal to destroy their toxic properties whilst respecting their antigenic principles as in the case of diphtheria toxoid or anatoxine (RAMON, 1925, GRASSET and ZOUTENDYK, 1933). The possibility of safely injecting high concentrations of anavenoms led to a rapid method of preparing antivenomous therapeutic sera, such as anti-*N flava* and anti-*B arietans* polyvalent serum used in previous experiments (GRASSET and ZOUTENDYK, 1932).

#### INJECTIONS OF *N flava* ANAVENOM TO RABBITS

Three rabbits were injected with 2 c.c. and three others each received 4 c.c. of *N flava* anavenom corresponding respectively to 20 and 40 mg of original *N flava* venom, *i.e.*, 57 and 114 M.L.D. for rabbits. (Detoxication of 10 per cent venom solution was obtained after a month of treatment with 0.8 per cent formal at 37° C. For detailed study on anavenoms, see GRASSET (1945). All injections were given intravenously in the volume of 10 c.c. No signs of intoxication or discomfort were observed in the rabbits so treated. Details of the respective blood sugar determinations of the four sample bleedings carried out on each rabbit before injection, 2 hours, 4 hours and 24 hours later are given in Table X.

From these results it appears that only a slight increase in the blood sugar of the rabbits is observed during the 4 hours following the injections of *N flava* anavenom with the exception of Rabbit 58 (40 per cent). No relation can be observed to the quantity of anavenom injected.

The fourth sample of blood taken the following morning (22 to 24 hours) shows in all the cases, including Rabbit 58, a subsequent drop practically down to the original blood sugar level, or a hypoglycaemia.

TABLE X.

BLOOD SUGAR CONTENT IN MGL. PER 100 C.C.

Starved Rabbits.

Number	Weight in grammes.	Amount <i>N. ferox</i> anavenom injected intravenously	First bleeding before injection.	Second bleeding 2 hours later	Third bleeding 3 to 4 hours later	Fourth bleeding 24 hours later	Per cent. variation in blood sugar at bleedings.		
							Second.	Third.	Fourth.
54	2,000	2 c.c. (20 mg.)	85	90	100	90	+ 5.8	+17.4	+ 5.8
55	2,100	2 (20 )	95	98	110	90	0	+18.8	- 5.2
56	2,400	2 (20 )	75	88	70	85	-24.4	- 6.6	-48.6
57	1,850	4 (40 )	65	68	70	65	0	+ 7.4	0
58	2,380	4 (40 )	80	70	70	88	+10	+10	+10
59	2,750	4 (40 )	78	70	70	65	-6.6	- 6.6	-13.2

INJECTION OF *B. aristans* ANAVENOM TO RABBITS.

A similar experiment was carried out with *B. aristans* anavenom (detoxication of 10 mg. venom solution in saline treated with 0.8 per cent. formal for 40 days at 37° C.)

Four starved rabbits received an intravenous injection of 5 c.c. of this anavenom (50 mg. of the original venom, corresponding to 35.7 M.L.D. before detoxication) and without apparent discomfort. Four blood sugar estimations were carried out at intervals as in the case of *N. ferox* anavenom. As shown in Table X, after a certain blood sugar rise during the 2 hours after injection (Rabbit 63), this was followed by a rapid drop to normal levels by the 4th hour.

This moderate and short blood increase observed is comparable to that seen in the case of the venom-antivenene neutralized mixture.

Finally with a view to ascertaining the eventual glycaemic action of imperfectly detoxicated venom, two rabbits were submitted to the injection of 30 and 50 mg. of a *B. aristans* venom treated with 0.8 per cent. formal for 40 days instead of 0.8 per cent. as in the previous case.

The detoxication of the product was sufficiently advanced to result in the delayed death of these rabbits in 5 and 4 hours, i.e. same death time observed with one lethal dose of untreated venom (1.4 mg. or a quantity of venom twenty-one and thirty five times smaller in weight) as in the case of formalized venom.

Blood sugar determination carried out before injection 2 hours later and soon before death showed in the Rabbit 65 injected with 30 mg. a lowering in blood sugar from 50 to 30 mg. in 4 hours, and in Rabbit 63 injected with 50 mg., an increase of the blood sugar from 55 to 125 mg. per c.c. (127 per cent. increase). Another rabbit, Rabbit 64 was injected with 20 mg. of venom

TABLE XI  
BLOOD SUGAR CONTENT IN MG PER 100 CC

Starved Rabbits

Num-ber	Weight in grammes	Amount <i>B. arietans</i> - anavenom injected intravenously	First bleeding before injection	Second bleeding 2 hours later	Third bleeding 4 hours later	Per cent. variation in blood sugar at second bleeding	Per cent variation in blood sugar at third bleeding
60	2,800	50 mg (0.8% (formol))	95	95	105	0	10.5
61	2,300	50 " (0.8% " )	90	105	105	+ 16.6	+ 16.6
62	2,050	50 " (0.8% " )	105	105	120	0	+ 14.3
63	2,800	50 " (0.8% " )	60	100	60	+ 66.6	0
64	2,800	20 mg (0.4% formol)	30	90	70	+200	+133.3
65	2,200	30 " (0.6% " )	50	40	30	- 20	- 40
66	2,700	50 " (0.6% " )	55	90	125	+ 63.6	+127.2
67	2,180	50 " (0.6% " )	75	85	70	+ 13.3	0

treated with 0.4 per cent formol, 40 days' incubation at 37° C. As a result of the partial detoxication, the rabbit died with acute toxic symptoms after 4 hours. This was accompanied by a 200 per cent increase in blood sugar after 2 hours and 133.3 per cent soon before death.

Thus whilst the injection of completely detoxicated venom exerts a minute variation of short duration in the blood sugar content, the presence of small residual toxicity in improperly detoxicated venoms is reflected by disturbances in blood sugar either by significant increase or continual drop below normal levels prior to death of the animals.

A further test was made with the same venom referred to above, treated with 0.6 per cent formol, but after an additional period of 15 months incubated at 37° C. The product which was then quite atoxic, was injected intravenously into a rabbit, Rabbit 67, in a dose of 50 mg. As shown in Table XI, only a slight sugar increase was observed after 2 hours, with a return to normal after 4 hours.

Determination of the blood sugar in such conditions provides us, therefore, with a further control method of checking the atoxicity of anavenoms, and of detoxicated venom derivatives.

#### CONCLUSIONS

The present investigations on the hyperglycaemic action of *N. flava* and *B. arietans* venoms corroborate the findings of BERTRAND and VLADESCO on this recently evidenced property of venoms, with the following contributions —

The *intravenous* injection of the respective venoms to starved rabbits in doses fatal within a few hours provoked a rapid, sharp and continued rise in the blood sugar content, reaching before death a maximum increase in the blood sugar from 100 to 400 per cent.

The *subcutaneous* injection of the same venoms to starved rabbits is reflected first by a moderate increase in the blood sugar. In non-starved rabbits an appreciable hypoglycaemia is first observed. This initial phase is followed in starved as well as non-starved rabbits by a sharp rise up to the time of death as in the case of intravenous injection.

The hyperglycaemic action of snake venoms appears to be linked with their toxic properties, as the injection of considerable doses of these venoms, either after the neutralization by specific antivenomous sera, or after their transformation into atoxic, yet antigenic derivatives (anavenoms) does not result in any further such hyperglycaemic action.

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## PRIMARY SPLENIC ABSCESS

BY

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The purpose of the article is to draw attention to primary splenic abscess as it is not usually easy to diagnose. In my experience it is not a common condition, but is encountered from time to time. In the last 3 years I have met with three cases. Others dealing largely with Native practice may encounter it more often, or again, some may never have seen it.

It is seen, as far as I am aware, only in the Native races. It is probable that the diagnosis may be overlooked in many cases of left basal pneumonia or other acute inflammatory conditions of the left lung or left upper abdomen. Unless it is treated by drainage the abscess will rupture into the abdomen with fatal consequences.

One of the earliest references to this condition and an attempt to elucidate its causation was that by A. C. WALLACE (1922), working in Broken Hill, Northern Rhodesia. He recorded a large number of cases seen more or less together in an outbreak. These cases had an acute influenza-like onset with pyrexia and marked constitutional disturbances. There was pain over the splenic region with a tender mass.

The course of the illness is of relatively short duration, the condition becoming more serious as the illness progresses. Unless the pus in the spleen is drained, the inflammatory splenic swelling increases in size and within 2 or more days a bulging in the splenic region or a generalized swelling of the abdomen becomes obvious. The temperature is moderately or markedly elevated, the pulse rate is accelerated, and there is a polymorphonuclear leucocytosis of varying severity. Owing to the position of the tumour, which may be likened to a sac of pus, the left dome of the diaphragm becomes elevated. This can best be demonstrated on fluoroscopy or radiologically. In the case here reported, a considerable elevation of the left dome of the diaphragm was evident. Such an elevation was also present in my last case; the patient, however, died later. I had probably delayed too long in advising surgical intervention. This was because a splenic abscess is not always, at first, easy to distinguish from a left basal pneumonia (which it may simulate very closely),

\* My thanks are due to Colonel A. P. MARTIN, O.B.E., M.D., Medical Director of Southern Rhodesia, for his kind permission to publish this paper.

an acute abdominal condition or an acute perisplenitis occurring in a malarial spleen presumably due to infarction. The latter condition is worthy of mention as it is by no means infrequent in the Native of tropical Africa, owing to the high incidence of malaria. Here the patient also experiences severe pain in the splenic region, accompanied by some degree of fever and in most cases by slight constitutional upset. The pain may persist, too for a few days. Thus one may be chary of inserting a needle into the spleen to confirm a diagnosis of splenic abscess. This means of differentiation is important because splenic aspiration is a dangerous procedure. Therefore before needling one would like further evidence of the presence of frank pus in the spleen. This can be obtained by noting the position of the left dome of the diaphragm by X-ray. In primary splenic abscess it is immobile and elevated, reaching a level higher than the right dome. Thus any case in a Native in which there is a sudden onset of pain and a tumour in the left side of the abdomen, both of a relatively short duration (3 or 4 days) the question of primary splenic abscess should be seriously considered. The diagnosis can be confirmed, almost certainly by the presence of an elevated left dome of the diaphragm. In perisplenitis there is no such elevation of the dome of the diaphragm.

It may be argued that an amoebic abscess in the left lobe of the liver may also cause such an elevation. In amoebiasis, however a tender splenic tumour will not be felt on examination. Further amoebic abscess of the left lobe of the liver causing elevation of the left dome of the diaphragm only is extremely rare. In my experience, almost all liver abscesses appearing on the upper surface of the liver affect the right lobe.

On aspiration, the pus is brownish or a chocolate brownish colour and of a fluid character. It closely resembles the pus removed from a liver abscess. This is the only resemblance one sees to a liver abscess. This colour is no doubt due to the large amount of blood which is normally present in the spleen and which has undergone degenerative changes.

Primary splenic abscess is quite different from amoebic abscess of the spleen, primarily because its course is so much more rapid. Secondly amoebic abscess of the spleen is usually found in association with amoebic lesions in other viscera, especially in the liver and bowel. FRANK (1944) records a case of amoebic abscess of the spleen, the liver being unaffected, but cysts of *Entamoeba histolytica* were found in the stools.

The aetiology of primary splenic abscess is not known. The fairly rapid course of this condition would suggest that the blood supply to the spleen had been cut off because the whole splenic tissue is replaced by a sac of fluid-like pus and no residual splenic tissue can be detected, the whole being enclosed in the capsule. In the first case of primary splenic abscess I encountered, at autopsy the pus was sterile on culture. No amoebic organisms were found. In the present case a mixed growth of staphylococci and streptococci was cultured, but amoebae were not detected.

Out of the forty-nine cases of splenic abscess seen by WALLACE, three were women and the rest adult males. My cases were all males. At first it was thought by WALLACE, that spirochaetes of the relapsing fever type might be the cause, but subsequent investigations failed to confirm this. Similarly, typhoid was excluded, as well as typhus, undulant fever and syphilis. In WALLACE's cases no amoebae were found in the pus. A spore-bearing bacillus, presumably gas-forming, was found in one of his cases. This is interesting as in the case I am reporting, a large gas shadow in the abscess was seen in the radiogram.

Whereas in my few cases the whole of the spleen was replaced by pus, WALLACE reports cases at autopsy, where some of the spleens showed macroscopically a marked similarity to multiple infarction, whilst, in others, the whole organ was affected. This brings to mind the case of a young Native woman, aged about 25, who was admitted to hospital with severe pain over the splenic region. She was collapsed and died within a few hours. At autopsy, an extensive and recent infarct of the spleen, extending to the capsule, was found. It thus seemed possible to me that splenic abscess is a later stage of infarction and one in which the whole blood supply of the spleen is cut off, liquefaction with colliquative necrosis subsequently developing.

In its simplest form perisplenitis is merely a small infarct occurring at the periphery of the spleen. The finding, at autopsy, of these infarcts in malarial spleens is of very common occurrence. A large infarct, on the other hand, may be accompanied either by severe pain and shock with possible death, or the infarct area may undergo liquefaction with the development of a primary splenic abscess. Thus the first essential for splenic abscess may be the presence of an enlarged malarial spleen which may undergo infarction at a later stage.

#### CASE HISTORY

The patient was a young Native male, aged about 24 years, from Portuguese East Africa. He was well until 4 days prior to his admission to hospital on the 19th October, 1944. His first symptom was severe abdominal pain which, though continuous, was aggravated by movement. He soon became aware of a mass on the left side which rapidly increased in size and was extremely tender. His bowels were normal. As far as we could determine, there was nothing of note in his past history. His diet—consisting of mealie-meal with meat and oranges twice a week—was fairly satisfactory for a Native.

His temperature, on admission, was  $101^{\circ}\text{F}$ , and pyrexia of this severity continued for a long period. He was wasted and his conjunctivae were pale. His tongue was slightly furred and the corners of his lips slightly fissured. His skin was dry and desquamating over the limbs and trunk. Follicular seborrhoea was noticed over the naso-labial folds. The trachea was displaced to the right. The apex beat was in the third left intercostal space, 2 inches from the mid-sternum. A pre-systolic triple rhythm was audible. There was no movement of the left side of the chest and stony dullness and absence of breath sounds



was noticed at the left base. The spleen, which was very tender, was enlarged to about four fingers breadth below the left costal margin.

In view of the findings at the left base, a diagnosis of pneumonia and pleurisy was made. The patient, however, failed to improve and the lump in the abdomen increased considerably within the next 48 hours, reaching to below the umbilicus.

His chest was X-rayed, and this showed a considerably elevated left dome of diaphragm with what appeared to be an abscess cavity below the fluid level. On the 4th day it was decided that the patient had a splenic abscess. This was aspirated and brownish pus was withdrawn, thus confirming the diagnosis. Surgical opinion was sought and the abscess was drained, 3 pints of pus being removed. The pus was examined for amoebae, but with negative results. The culture showed nothing of note beyond a mixed growth of streptococci and staphylococci. The examination was repeated, but still no amoebae were found. In the stool ova of hookworm and *Trichuris trichiura* were present. The urine contained *Bilharzia haematolum*. The Wassermann reaction was negative. Unfortunately the patient did not make the recovery anticipated because the abdominal incision was made too high and not sufficiently dependent for the abscess to drain. He was given a transfusion a few days later and it was only 1 month later that his temperature subsided and one began to be hopeful. His diet was now very rich in first-class protein, vegetables and vitamins, but in spite of that he did not make any satisfactory progress. His blood count rose but only slightly after a month, but then began to drop slowly. He was given another two transfusions, but he died on 5th January 1945. It was clear to us that he still had a big residual sac of pus which we had failed to evacuate. A radiogram, taken earlier in January showed the left dome of the diaphragm still elevated (see Plate).

At autopsy the body was extremely emaciated with well marked signs of pellagra. There was pallor of the viscera due to anaemia. No abnormality was seen in the lungs, heart, liver, kidneys or brain. Dense perisplenic adhesions were found, and when these were separated, a large abscess was revealed containing about 8 ounces of greenish yellow thick lumpy pus which had a bad odour. The wall of the abscess was carefully examined for amoebae, but without success. The pus itself was cultured and showed colonies of mixed organisms. The large intestine did not reveal anything in the mucosa suggestive of amoebiasis. The mucosa looked atrophic, but this is a common finding in advanced pellagra. Sections of the abscess wall showed an acute, or chronic inflammatory reaction of pyogenic type.

#### SUMMARY

1. The clinical features of primary splenic abscess are described, the main ones being a rapid onset and febrile course with pain over the splenic region and a tender splenic mass which rapidly enlarges.



RADIOGRAM SHOWING ELEVATION OF LEFT DOME OF DIAPHRAGM STILL PRESENT A WEEK PRIOR TO DEATH

- 2 Elevation of the left dome of diaphragm is described in the case reported
- 3 The illness is likely to be mistaken for left basal pneumonia, empyema or perisplenitis
- 4 It is suggested that primary splenic abscess may, in the first instance, follow massive infarction of the spleen due to thrombosis of the splenic artery.

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# MALARIA IN THE NATIVES OF NEW GUINEA

BY  
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AND  
J MAIER, MAJOR, MC, AUS

It has long been known that malaria is hyperendemic in Papua and the Mandated Territory of New Guinea. However, few attempts have been made to measure its intensity. We present here the results of smear and spleen surveys from nine different areas. Over 2,000 natives were examined and the classified results are presented in Tables I to V. The localities are shown on the accompanying map.

While all of the areas studied must be considered hyperendemic for malaria, there is considerable local variation, both in the intensity of transmission as measured by parasite rates and plasmodiometric indices,† and in the

\* The personnel of the Australian New Guinea Administrative Unit (ANGAU) deserve special thanks for permission to carry out this work, and for their invaluable assistance.

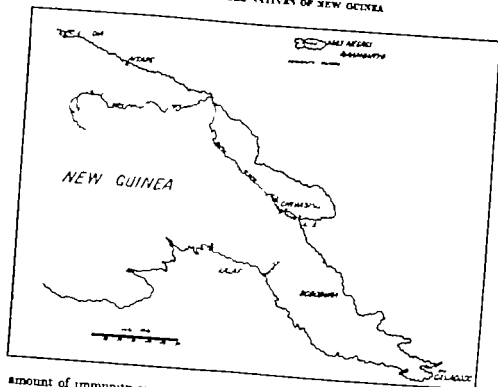
We are indebted to Major T C BACKHOUSE, 2/7th AGH, for permission to use one of the parasite surveys of Butibum village, and to Major F J DY, HQ, USAFFE, for permission to use his survey of Gemadodo.

We are also indebted to the commanding officers and personnel of the 4th, 5th, 17th, 24th, 27th and 28th Malaria Survey Units, for their assistance. T/3 AMOS H TREADWAY, T/5 ARNE W SEVERSON, and PFC BARNEY A CYBERSKI, supported the work with exceptional effort.

† Proposed by PARROT, CATANEI and AMBIALET (Observations parasitologiques sur le paludisme en Algérie II Le paludisme épidémique—*Arch. Inst. Pasteur d'Algérie* (1940 Dec, 18 (4) 402-440) as a means of measuring the intensity of the infections by separating them into six categories. We have arbitrarily separated our parasite counts per 500 white blood corpuscles into these categories as follows—

Category	Parasites/500 W B C
1	1-2
2	3-9
3	10-19
4	20-49
5	50-149
6	150 and over

The plasmodiometric index represents the average category of the positives in any group



amount of immunity as measured by the spleen rates and sizes. It is difficult to compare our results with pre war figures because of the different conditions and localities studied and because many of the previous data have been presented without reference to age groups or spleen size the notable exception being the studies of HEYDON (1923) at Rabaul.

The importance of recording age groups and sizes of palpable spleens can be demonstrated by a comparison of the findings on Butubum Village (Lae) and Mokerang Village (Admiralty Islands). Both villages are hyper endemic, and a lumped spleen rate for children up to 15 years would indicate that Mokerang, with 94 per cent. palpable is carrying a considerably heavier burden of malaria than Butubum with 68 per cent. palpable. Actually the reverse is true, as a study of the chart show (Fig 1). In Butubum Village it is seen that children less than 2 years old have larger spleens and a higher per cent. palpable spleens than any other age group and that in the succeeding age groups there is an even decrease in spleen size and per cent. palpable spleens. This means that immunity begins very early and is almost completed by the time the 11 to 15 year group is reached. In Mokerang, however the peak of spleen size and per cent. palpable spleens is not reached until the 6 to 10 year group the decrease in these factors beginning at 11 to 15 years, an age when immunization is nearing completion in Butubum. Since immunity to malaria

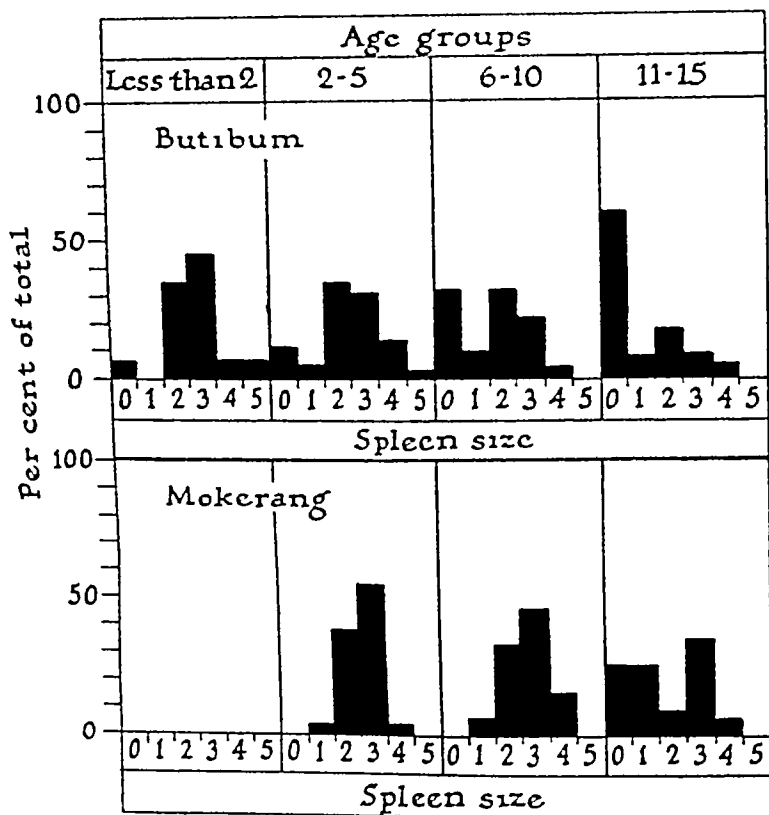


FIG. 1. Distribution of spleen size in two native villages

can only be acquired by repeated infection, it follows that a population which acquires its immunity more rapidly than another has received a larger number of infections earlier. That is, infection with and transmission of malaria is more intense in Butibum than in Mokerang.

The best measure of malaria transmission is a combination of the study of anopheline prevalence and the determination of infection rates. Unfortunately, adult *Anopheles punctulatus* is difficult to catch in numbers, and in our studies only those from Lalapipi have been dissected in number.

It has been demonstrated (HACKETT, 1932) that as transmission varies, the relative prevalence of the three species of malaria parasite varies in the following manner. *P. falciparum*, an intense infection of relatively short duration, assumes dominance when transmission is intense. As transmission decreases, *falciparum* tends to recede, *vivax* becomes dominant, and *malariae* increases. Comparing Butibum and Mokerang Villages, Tables I and II show that in Butibum the per cent positives and plasmodiometric indices are considerably

TABLE I New Guinea. Iar, Bushman Village; B

NEW GUINEA. Loe, Bushman Village; B.

Spleen clams.

Age group.

Total studied.

0 1 2 3 4 5

Less than 2 21

2-3 74

6-11 26

11-16 19

Less than 2 13

2 45

6-11 67

11-16 8

Feb 1944

Age palpable No clams given.

November 1944

Less than 2 12 1 7

2-3 30 5 12 5 18 36 13 31 6 14 1 2 43

6-10 91 16 22 8 1 27 35 17 21 2 4 22

11-16 44 27

The following symbols denote:

Pneumodermic index

Number of spleens examined  
Spleens not returned.

No No No No No No

P. mae.

T G T G T G

P. falciparum

P. malariae

M. and m. f.

tooth

L. incriminated

Pneumodermic index

Pouch

P. mae.

P. falciparum

P. malariae

M. and m. f.

tooth

L. incriminated

Pneumodermic index

Pouch

P. mae.

P. falciparum

P. malariae

M. and m. f.

tooth

L. incriminated

Pneumodermic index

Pouch

The following symbols denote the

percentage of parasites

found in parasites

TABLE I NEW GUINEA. IAR, BUSHMAN VILLAGE; B

[illegible]

The following symbols denote the number of

$G$  = Gametocyte

$T$  = Trophozoite



higher than in Mokerang, while Fig. 2 shows the predominance of *falciparum* in Butubum and of *vivax* in Mokerang.

The most complete survey was carried out on the two native villages Uruta and Isapepi, near Lalapipi, at the mouth of the Lakemba River in Papua. This was done in connection with an experiment in which it was desired to have an experimental village and a control village under as nearly identical conditions as possible. These villages, about 1 mile apart, were both located on a narrow sandy area separating the beach from an extensive sago-palm swamp. This swamp provided extensive breeding areas for *Anopheles*

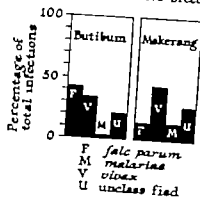


FIG. 2. Relative prevalence of the three species of malaria parasites in two native villages.

*punctulatus molluccensis* which was present in great numbers. This abundance of adult anopheles is unusual in New Guinea. It was first noticed in the area by Captain ATHERTON, A.A.M.C (1943) in September 1943 and was confirmed by us in February and September 1944. These dates cover both the wet North-west season (November to April) and the dry South-east season (May to October). Our survey done in September near the end of the dry season indicates a large amount of residual malaria in the population with small amount of transmission actually going on. (Table III and Fig. 3.) Anopheline infection rates were very low—0.06 per cent sporozoites and no oöcysts for *A. punctulatus molluccensis* (one gland in 1737 dissections) and no sporozoites and 0.4 per cent oöcysts in *A. subpectus* (one gut in 263 dissections). These rates are so low that it is probable that malaria was maintained at this time only by the enormous number of anophelines. On the other hand, the low mosquito rates of infection are unexplained.

In parasite and spleen rates the two villages are very similar (Table III and Figs 3 and 4). They are alike in per cent. positive, and in the incidence and size of palpable spleens in the different age-groups. Also, the relative incidence of *falciparum* and *vivax* is similar in the two villages. This is to be expected in view of the practically identical conditions and gives an idea of

### TABLE III

The following symbol denotes the number of gametocytes included from mixed infections

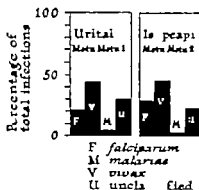


FIG. 3. Relative prevalence of the three species of malaria parasites in two neighbouring villages.

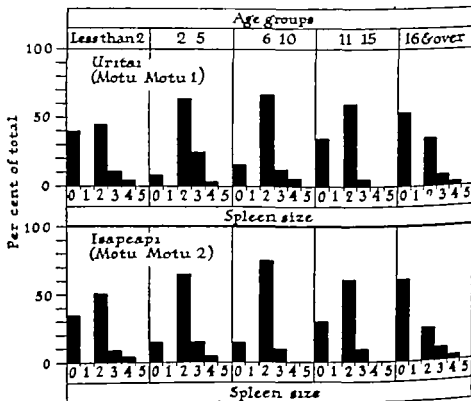


FIG. 4. Distribution of spleen size among individuals of two neighbouring villages.

how accurately a malaria situation can be measured and what minor variations can be expected under the same natural conditions

That some variation may be expected at different times of the year is shown by the surveys on Inonda village, near Dobodura (Table IV). Of course, the extent of this variation depends on the variation in climatic factors affecting transmission—chiefly temperature and rainfall. At none of the localities studied does the temperature vary enough to have any effect on transmission, so fluctuation in rainfall here is the only factor. At Inonda, the smear surveys indicate a higher rate of transmission in February than in October. This difference appears in the relationship of *falciparum* to *vivax* (Fig 5), in

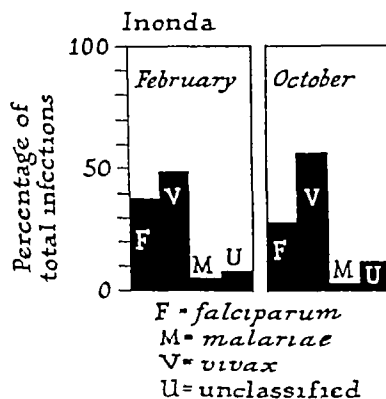


FIG 5 Relative prevalence of the three species of malaria parasites in two surveys of the same village

the per cent positive smears, and in the plasmodiometric index (Table IV). While the difference between the two surveys is not great, it is statistically significant. The difference may be accounted for by the fact that the February survey was taken at the height of the wet season, while the October survey was taken at the end of the dry season, when mosquito breeding was at a minimum.

These surveys show that hyperendemic malaria can be maintained in New Guinea in the absence of "man made" breeding places—wheel ruts, ditches, and borrow pits—since nearly all of these villages are well away from military areas and roads where such breeding places exist.

#### METHODS

Giemsa-stained thick smears were examined. A slide was not considered negative until an area which included 500 white blood cells had been covered. Splenic examinations were routinely carried out with the subject standing, and the results classed as 0-5 according to HACKETT's (1944) recommendations.



Doboduru, Inonda Village 22nd February, 1944

Less than 2	13	1	3†				2	07			3	10	5	3*	2	2†		VF, 1 VM, 2 MF, 1	4.9	100
2-5	7						1	17	2	33	3	50			1*	2		VF, 3	1	4.8
6-10	37	2	5	1	3	14	38	13	35	6	10	1	3	37	13	0†	6	VF	7	3.4
11-15	3					3	100							3	1	1				4.0
16 and over	11													11					1	2.4

Inonda Village, 20th October, 1944

0-10	45										0	17	3	7	5	1	1	VF, 1	5	2.9	71
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Milne Bay Gamadodo Village, 23rd May, 1943 (courtesy Major F. J. Day)

0-14	50	100 per cent positive—Spleen classes 3 and 4										40	10	3	0	17	1	8	5	V Γ, 3 F M, 2 V M, 1			68
15 and over	50											50	1	0	8	1	0	0	F M, 1			26	

The following symbols denote the number of gametocytes included from mixed infections \* 1 † 2

TABLE V. NEW GUINEA. Hollandia, Tobadi Village.

TABLE V. New Guinea. Hozomeia, Tobad Valley.

Age group	Spleen class										Parasites														
	Total studied		Yes		No		Yes		No		Number of spleens examined		Number not examined		P. tricus		P. falciparum		P. malariae		Mixed infection		Unidentified		% Positive
															T	G	T	G	T	G					
Less than 3	21				4	37	2	10	3	27	2	18			3										
3-5	28	1	4	8	10	10	38	7	27	2	12				3		2	1					2	30	36
6-1	22	2	15	3	23	3	18	4	21	3	15				1		1	1					2	21	29
11-15	11																								
16 and over	32																1						1	18	18
																							3	20	18

Adapta. Correl. ratios between Radius and Nuclei threat.

Less than 3	10																										
3-5	12		1	16	32	78	54	8	11															48	67		
6-1	78																							VP 2	9	31	73
11-15	11																							6	21	63	
16 and over	84																							1	20	26	
																								2	29	17	

The following symbols denote the number of examinations conducted.

This table being applied decreases the number of gametocytes included from mixed infections.

Exception to this was made in the surveys of Uritai and Isapeapi, where the observer had the patient lie down and did not differentiate between spleens palpable on respiration and those reaching as far as half-way to the umbilicus

#### SUMMARY

1 The hyperendemicity of malaria in New Guinea has been determined by parasite and spleen surveys. In one village transmission was so intense that the maximum incidence of palpable spleens was found in the 0-2 year age group. Older children had sufficient immunity to cause the percentage of palpable spleens to decrease.

2 The importance of classifying the results according to age groups is emphasized by a comparison of two villages. The village with a spleen rate of 68 per cent in children actually had more malaria than did the village with a rate of 94 per cent.

3 Combined parasite and spleen surveys carried out simultaneously on two similarly situated villages gave almost identical results. Thus a properly done survey can be a very accurate measure of the amount of malaria present and of current transmission.

4 The plasmodiometric index which measures the parasite load of a population is a useful adjunct to the standard survey.

5 Seasonal variation in transmission was demonstrated in one locality. It was apparently connected with the rainfall.

6 Since hyperendemic malaria is maintained without the addition of artificially created breeding places in all coastal villages, it is important to pay attention to natural breeding places as well as to those created by military occupation.

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# DDT SPRAYING INSIDE HOUSES AS A MEANS OF MALARIA CONTROL IN NEW GUINEA

BY

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AND

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The killing of adult anophelines as a means of control of malaria has long been in use the world over LEPRINCE (1916) first used it extensively against *Anopheles albimanus* during the construction of the Panama Canal He later recommended it in the control of *A quadrimaculatus* in the southern United States (1926) With the development of pyrethrum solutions in kerosene for spraying, the destruction of adults has been routinely carried out in many places A summary of this work is presented by JOBBINS (1941) Controlled experiments in India by RUSSELL and KNIPE (1943) during four seasons of transmission demonstrated that there was a marked reduction in the amount

\* We are greatly indebted to the Australian New Guinea Administrative Unit (A N G A U) for making this study possible and for furnishing transportation To Major THOMSON, Captain ROSS, and Mr ED HICKS of that organization we are particularly grateful for their constant help

Major A M HARVEY, M C, A U S, assisted in the organization of the study and was a member of the unit during the first part of the study

of malaria through the destruction of mosquitoes shortly after they had fed on gametocyte carriers

The introduction of DDT sprays which have a residual effect of several months or more eliminates the necessity of weekly spraying. It does not alter the fact that the success of inside spraying is dependent upon the habits of the particular anopheline responsible for the transmission of malaria in a given place. Residual DDT can only reduce the number of infected mosquitoes if they rest on the sprayed walls either just before or after feeding on the gametocyte carrier

Both *A. punctulatus punctulatus* and *A. p. woluccensis* usually spend the day resting in some cool moist area away from houses. This might be expected since survival in the laboratory is greatest when the adults are kept in very moist surroundings. However adults have been taken resting on the walls of native houses during the day in such widely separated localities as Cairns, Australia Lalapipi in Papua Nadzab Salamaua and the Admiralty Islands of Australian Mandated New Guinea. Of equal significance are scattered observations in Australia and New Guinea that unfed females will frequently rest on the walls or furniture of a house, or the sides of tents at night before feeding. They may remain on the same spot for over an hour in either dark or lighted places. It is clear that a large proportion of those anophelines which feed on the gametocyte carriers at night would be subject to the action of DDT

Data on the use of residual sprays in the control of malaria have been limited to the direct effect on the mosquito populations. The reduction of malaria by this method in the Australasian region has not been determined. This paper reports studies on the changes in the amount of malaria following DDT spraying of the houses of a native village with simultaneous studies on an untreated village. For this purpose the villages of Motu Motu 1 (Uniai) and Motu Motu 2 (Isapenpi), on the southern coast of Papua, were selected. They are about 150 miles west of Port Moresby

The presence of great numbers of *A. punctulatus woluccensis* was first discovered by ATHERTON at nearby Lalapipi in June, 1943 (Unpublished observations.) The many enlarged spleens in the children, and the similarity of the two villages in location also made this an ideal place for a controlled study

The two villages are about 2 miles apart on a coastal strip which has been broken off from the mainland by the meandering course of the Lakekamu river. They are comparable in every way except size. Motu Motu 1 (the treated village) being twice as large as Motu Motu 2 (untreated). The coastal

All of these observations or records were either made or confirmed by one or another of the authors. Among the entomologists who first made such observations are Captains H. BROWN, A. M. DONALDSON, O. H. GRAHAM, M. S. FERGUSON, S. E. SEXTON, A.U.S., and Captain ATHERTON, A.A.M.C.

part of the island consists of a flat sandbar only a few feet above sea level overgrown with grass, scrub, and coconut palms. The inland border adjoining the river is covered with nipa and sago palm swamp. This swamp also extends for many miles inland and along the coast, and presumably provides an excellent breeding ground for *A p moluccensis* and *A subpictus* which were present at all times.

The native thatch houses are built on piles 6 to 8 feet above the ground, which is kept clear of all vegetation except for a few trees. The floors are made of rough hewn planks laid loosely together, while the walls are of woven palm leaf, thatch or bamboo strips. Adult anophelines may be taken in numbers underneath the houses throughout the night. The natives spend most of the night sleeping on the floors above. Thus we might expect a large measure of control to be brought about by the destruction of adults entering the houses at night, although incidental infections may be acquired by sleeping in gardens and by nocturnal visits between villages.

The transmission of malaria in a given place is related to the number of anophelines present and the proportion infected. As later figures show, *A p moluccensis* and *A subpictus* were extremely prevalent, but the mosquito infection rates are the lowest reported in New Guinea. ATHERTON thought that this was due to a shorter life in the anophelines here, and later observers thought it due to strong south-east winds. However, the mosquito infection rate was still low in January, 1945, when the sea breeze was minimal. Thus the results summarized in Table I are unexplained. The extreme prevalence of anophelines in this area maintains the hyperendemicity of malaria.

TABLE I

DISSECTIONS OF ANOPHELES IN AND NEAR VILLAGES OF MOTU MOTU 1 AND 2

Time	<i>Anopheles punctulatus moluccensis</i>				<i>Anopheles subpictus</i>			
	Salivary glands		Stomachs		Salivary glands		Stomachs	
	No dissected	% Pos	No dissected	% Pos	No dissected	% Pos	No dissected	% Pos
Sept 1943								
Oct 1943	500	0.0	500	1.2				
(ATHERTON)								
Sept 1944	1737	0.06	1000	0.0	263	0.0	200	0.3
Jan 1945	285	0.00	93	2.1	224	0.0	47	0.0
Total	2522	0.04	1493	0.5	487	0.0	247	0.4

## COMPARISON OF TWO VILLAGES.

We had found in previous malaria surveys in New Guinea that careful spleen measurements and thick smear examinations would, when classified into age groups, give an accurate differentiation of the amount of malaria in any particular community even though all of the villages studied had been hyper-endemic (HARSTOCK BANG MAIER, 1947). Because of the excellent census taken by A.N.G.A.U. (Australian New Guinea Administrative Unit) we were able to examine almost every inhabitant of Motu Motu 1 and 2 and maintain a complete record of the age, sex and dwelling of each individual.

The results of this survey indicated that the two villages had approximately the same amount of malaria both had a low mosquito infection rate and an equally high prevalence of adult anophelines.\*

## APPLICATION OF DDT

All of the 126 houses in Motu Motu 1 were sprayed on 8th or 9th September 1944. Only the interior of the houses was treated, the spray being applied to walls, partitions, and the adjoining edge of the floor and roof. Some houses had very low walls in which case part of the roof was also sprayed. The DDT was dissolved in kerosene to make a 4 per cent. solution, and this was applied in a fine mist by a hand operated knapsack sprayer at an estimated rate of 100 mg DDT per square foot.

## RESULTS

Before deciding the effect of the DDT spraying, it was important to determine what happened in the untreated village, and in the general surroundings. During the 4 months intervening between the experiment and the follow up study the strong south-east winds died down and in January the occasional slight breeze came from the land. Adult anophelines were again plentiful. It is possible that the change in the wind allowed the mosquitoes

The amount of malaria was measured according to the plasmodiometric index proposed by PARROT, CATANZI and AMIELLET (1940) as a means of measuring the intensity of the infections by separating them into six categories. We have arbitrarily separated our parasite counts per 500 W.B.C. into these categories as follow:—

Category	Parasites/500 W.B.C.
1	1-2
2	3-9
3	10-19
4	20-49
5	50-149
6	150 and over

The plasmodiometric index represents the average category of the positives in any group

to remain at the source of their infection a little longer and thus an increase in transmission might be expected. Because of the low rate of infected anophelines, our dissections are not numerous enough to establish this, but it is worthy of note that the true infection rate may have increased fifteen-fold without being demonstrable in our dissections.

As the parasite index is by far the most sensitive index in human populations of changes in transmission, this was carefully studied. The same observer who had counted and studied the parasites on the first survey studied them on the second. In Table II it will be noted that the percentage of individuals with positive smears increased in all age groups in the uncontrolled village, with an average increase of 17 per cent.

TABLE II  
PARASITE INDEX OF DIFFERENT AGE GROUPS IN MOTU MOTU 1 AND 2  
(Before and after DDT treatment)

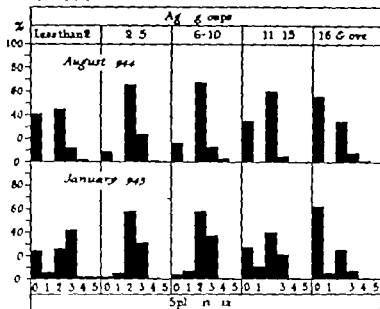
Age	Motu Motu 1 (Percentage treated)			Motu Motu 2 (Percentage untreated)		
	Aug	Jan	Difference	Aug	Jan	Difference
Less than 4 months		40			•100	
4 months to 1 year	60	68	— 1	60	91	+ 30 ± 10
2 to 5 years	62	53	— 9 ± 8 4	64	74	+ 10
6 to 10 years	40	42	+ 2	43	45	+ 2
11 to 15 years	32	25	— 7	26	49	+ 23 ± 10 3
16 years	22	16	— 6	14	32	+ 18 ± 5 3
Average			— 2			+ 17

• One individual

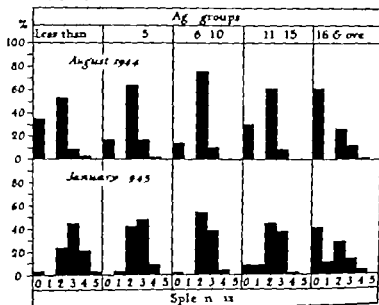
There was also an increase in the splenic index in all age groups (see Diagram). Thus it is likely that the DDT was applied during a season of low transmission and so worked against a naturally occurring cycle.

In the experimental village (Motu Motu 1) there was a slight but statistically insignificant decrease in the parasite index of all but one age group. The recorded splenic index is slightly increased, but it is likely that there was an equal decrease in size which is masked by the fact that two different observers did the surveys in September and in January. The second observer felt spleens with the individual standing, or occasionally sitting relaxed in a chair, while the first examiner had all the subjects lying down. The important point is that the first observer found splenic size the same in both villages in September,

Motu Motu 1



M u Motu 2



while the second observer found spleens about 50 per cent larger in January in Motu Motu 2, the untreated village

In previous reports we have indicated that when transmission is reduced the percentage of falciparum infections decreases. This would not occur within 4 months in a native population which is not on atebirin suppression, for uncontrolled falciparum malaria continues to relapse for as long as 4 months.

The plasmodiometric index is a numerical expression of the parasite load present in the blood of positive individuals (See footnote, page 812). Since the number of circulating parasites in the blood is dependent on the activity of the reticulo-endothelial system, the plasmodiometric index is a particularly good measure of the increasing immunity conferred by repeated attacks. This immunity is little changed in 4 months, and thus no change is to be expected in the plasmodiometric index of the two villages within this period of time. None was found (Compare Tables IX and XI).

### MOSQUITO STUDIES

If the application of DDT was still effective at 4 months, there should be fewer mosquitoes found in houses of the experimental village in the afternoon when they would have been on the walls longest. Table III presents the results of collections on the same days in Motu Motu 1 and Motu Motu 2, and in two villages to the east and to the west of the areas studied. Each house was searched by two observers working together, catching all anophelines.

TABLE III  
HOUSE COLLECTIONS IN FOUR VILLAGES

Lalapipi (Untreated)			Motu Motu 1 (Treated)			Motu Motu 2 (Untreated)			Kukupi (Untreated)		
No of houses	Number of Anophelines		No of houses	Number of Anophelines		No of houses	Number of Anophelines		No of houses	Number of Anophelines	
	Total	Per house		Total	Per house		Total	Per house		Total	Per house
3	145	48.3	2	10	5						
			6	1	0.2						
			10	20	2.0	4	70	17.5			
			2	1	0.5	4	120	30.0			
			3	5	1.7						
						4	101	25.0	4	68	17
3	145	48.3	23	37	1.7	12	291	24.2	4	68	17



found in the corners and crannies. Frequently the anophelines would be found resting on earthenware jars, on piles of clothing, boxes or other objects which had not been sprayed the first time. Despite this chance to escape the effect of DDT there was a marked reduction in the number found in houses of Motu Motu 1 the treated village.

It was thought that a difference in breeding between the two villages might account for this difference. Although the breeding was not located either in or around the villages, no difference in the terrain or available breeding places was observed. It is unlikely that of the four villages under identical conditions this one would have a decreased amount of breeding. This was further indicated by simultaneous collections of adults at night under the houses of the two villages. The two collectors changed posts to eliminate the personal factor. A difference significant only of chance variation was found. (See Table IV.)

To summarize the effect of the DDT while the uncontrolled village had a significant increase in the parasite rate in all age groups and an increase in spleen size, the experimental village had a slightly decreased parasite index and no true increase in spleen size. Thus we conclude that through a killing of adult anophelines either just before they were to feed on carriers or just

TABLE IV  
NIGHT COLLECTIONS OF ANOPHELINES NIGHT  
(Both Sex native children)

Time.	Motu Motu 1 (Treated).	Motu Motu 2 (Untreated).
8.30 to 9.30	63	94
9.0 to 10.0	78	89

TABLE V  
DAYTIME COLLECTIONS OF ANOPHELINES

Place collected.	<i>A. subpictus</i> .	<i>A. punctulatus</i> <i>malayensis</i>	Percentage <i>A. punctulatus</i> <i>malayensis</i> .
House walls, high	60	12	17
House walls, low	32	24	51
Overwood outside	3	20	87

after, the transmission had been reduced. The parasite rate was reduced instead of increased, and the spleens were kept from increasing in size.

#### PRECIPITIN TESTS ON BLOOD MEALS

The tendency of a given anopheline to feed on man may be measured by determining the percentage of wild caught females which contain human blood. Both *A. p. moluccensis* and *A. subpictus* were collected during the day as they rested in and around the houses of the untreated village. Freshly engorged specimens were crushed on a piece of filter paper. The blood stains were kept dry for several months, but were not always refrigerated. Antisera were prepared against human, dog, pig sera in rabbits by the injection of 1 c c of serum subcutaneously. The rabbits were bled after 10 days and the titration of the antiserum determined. Only those which when diluted one-third would produce a ring against a 1/1,000 dilution of the specific serum were used.

TABLE VI

NIGHT CATCHES IN MOTU MOTU I

Relative prevalence of *A. punctulatus moluccensis* and *A. subpictus*

	<i>A. punctulatus moluccensis</i>	<i>A. subpictus</i>
P M 8 40- 9 30	7	3
9 40-10 30	5	6
10 40-11 30	26	8
11 40-12 30	5	10
A M 12 40- 1 30	4	11
1 40- 2 30	4	10
2 40- 3 30	1	2
3 40- 4 30	1	5
4 40- 5 30	2	8
Total	55	63

Ring tests were performed in small tubes made from glass tubing. Antisera were made only against these three species because they are the predominant animals present in the villages. There were no cows or horses.

The results are presented in Table VII. The data in relation to the resting place of the mosquitoes are not presented because no significant difference was found in this small series.

The data confirm the tendency of *A. subpictus* to bite man and demonstrate that this species is just as anthropophilic, if not more so, than *A. p. moluccensis* in that area. The percentage of anophelines which fed on man has been calculated as percentage of total positive instead of total tested because it is

thought that the negative results were due almost entirely to poor specimens rather than to an untested source of blood meals. The results with *A. punctulatus moluccensis* agree fairly well with those obtained by HEYDON (1944) from the same area.

TABLE VII.

Species.	Number positive against			Negative.	Per cent. positive for mcn.
	Man.	Dog.	Pig.		
<i>A. punctulatus moluccensis</i>	4	1	7	21	13 + 5.9
<i>A. subpictus</i>	12	11	47	25	17 + 4.8

TABLE VIII.

SPLEEN SURVEY IN VILLAGES BEFORE DOT TREATMENT

Age group	Number of spleens examined	Spleen class.											
		0		1		2		3		4		5	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Mora Motu 1 31st August, 1944.													
Less than 2	84	23	41		5	43	7	12	1	2			
2-5	33	8	9		56	86	20	24	1	1			
6-10	119	19	18		61	68	16	19	3	3			
11-15	107	28	25		64	60	8	5	0	0			
16 and over	227	162	44		114	35	26	8	4	1			
Mora Motu 2 4th September 1944.													
Less than 2	32	11	23		17	53	3	9	1	3			
2-5	36	6	17		23	64	6	17	1	3			
6-10	89	8	14		45	76	6	10	0	0			
11-15	46	14	30		74	41	4	9	0	0			
16 and over	181	62	61		29	26	18	12	1	1			

Spleen enlargement is classified according to HACKERT's recommendations.

### DISCUSSION

These experiments were undertaken with the idea that malaria at Lalapipi and Motu Motu was transmitted entirely by *A. punctulatus moluccensis*. It became apparent during the course of the work that *A. subpictus*,

a vector of some importance in other areas, was present in fairly large numbers. It could be collected easily in the houses in all of the villages, freely came to bite at night within the village and was found infected in nature. Dissections were not extensive enough to determine the relative importance of the two anophelines in the transmission of malaria. Collection of adults during the daytime is not an adequate means of determining prevalence, for *A p moluccensis* rests outside much more frequently than does *A subpictus* which typically frequents houses. Collections even within the house have to be done with care, for *A p moluccensis* tends to rest near the floor much more than does *A subpictus*.

Flashlight collections by three people using themselves as bait showed that *A p moluccensis* was more frequent during the late hours of evening. On the other hand, *A subpictus* came more frequently to bite in the early morning hours, so the total for the two species was about the same. Thus *A subpictus* must be accepted as an important vector at Lalapipi and Motu Motu.

TABLE IX  
PARASITE SURVEYS IN VILLAGES BEFORE DDT TREATMENT

Age group	No of smears examined	Parasites									
		<i>P vivax</i>		<i>P falciparum</i>		<i>P malariae</i>		Mixed infections	Un-classified	Plasmodi-metric index	% Positive
		T *	G *	T	G	T	G				
Motu Motu 1, 31st August, 1944											
Less than 2	52	15	4	13	6**	1	1	V F, 2 V F, 1 F M, 1	5	3.9	69
2-5	79	30	2	9	2**	2	1		6	3.0	62
6-10	116	19	2	5	1	4	1		17	2.2	40
11-15	105	11	0	7	2	3	0		13	2.1	32
16 and over	318	25	3	9	6	0	0	V F, 1	29	1.8	22
Motu Motu 2, 4th September, 1944											
Less than 2	30	9	3	7	1				1	4.4	60
2-5	31	11	1	5	0				4	2.8	64
6-10	56	8	0	10	2				7	3.2	43
11-15	38	5	0	0	0	1	0		4	1.6	26
16 and over	125	9	1	3	1				6	2.0	14

\* T = Trophozoite G = Gametocyte V = *vivax* F = *falciparum* M. = *malariae*

The symbol \*\* denotes that one individual with gametocytes was included from mixed infections

## SUMMARY AND CONCLUSIONS

The application of DDT in kerosene to give a concentration of 100 mg. per square foot on the walls of houses in a native village in Papua, New Guinea reduced the transmission of malaria within this village in a 4-month period as evidenced by

1 The number of *A. punctulatus moluccensis* and *A. subpictus* found on the walls of the treated village was less than 5 per cent. of that found in neighbouring villages

2 While the parasite index and splenic index were increased in a neighbouring uncontrolled village, the parasite index was slightly decreased and the splenic index remained the same in the treated village.

3 There was no change in the amount of falciparum infection or the parasite density of the group having positive smears. This is to be expected since untreated falciparum malaria will continue to relapse for 4 months, and since there was no evidence of change of immunity during the 4-month period.

TABLE X

SPLEEN SURVEYS IN VILLAGES AFTER DOT TREATMENT

Age group	Number of spleens examined.	Spleen size.											
		0		1		2		3		4		5	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Motu Motu 1 (Treated); January 1948.													
Less than 2	31	12	24	3	8	13	26	31	42	1	2	1	2
2-5	30	2	2	4	8	50	55	27	31	1	1		
6-10	110	4	4	7	7	64	58	34	37	1	1		
11-15	92	25	27	10	11	37	40	19	21	1	1		
16 and over	59	161	62	14	5	64	25	17	7	3	1		
Motu Motu 2 (Untreated) January 1948													
Less than 2	32	1	3	0	0	8	24	15	43	7	21	1	3
2-5	31		0	1	3	14	42	16	46	3	9		0
6-10	61	1	2	0	0	33	54	34	39	3	4		0
11-15	47	4	8.5	4	8.5	21	45	17	33	1	2		0
16 and over	133	86	41	15	11	29	22	17	14	6	4		0

In Motu Motu 1 ten children were born after spraying three had palpable spleens, four had positive smears.

In Motu Motu 2, one child was born—spleen not palpable smear positive.

It was not possible to determine the effect of spraying on the infection rate of the mosquitoes since this rate was the lowest reported in New Guinea for *A punctulatus moluccensis*.

*A subpictus* was present in large numbers, came frequently to feed on humans, was found in the houses during the daytime, and was found infected

TABLE XI  
PARASITE SURVEYS IN VILLAGES AFTER DDT TREATMENT

Age group	No of smears examined	Parasites									
		<i>P vivax</i>		<i>P falciparum</i>		<i>P malaria</i>		Mixed infections	Un-classified	Plasmodi-metric index.	% Positive.
		T*	G*	T	G	T	G				
Motu Motu 1 (Treated) , January, 1945											
Less than 2	50	15	5	11	5	2	1	F+M, 1 F+V, 1	4	3.8	68
2-5	76	17	6	8	3	4	3	F+M, 1 F+V, 1	8	2.8	55
6-10	97	15	2	9	4	6	3	F+V, 1	10	2.7	42
11-15	83	9	1	5	3				7	1.7	25
16 and over	245	12	1	4	2	4	1	F+M, 1 F+V, 1	14	1.7	16
Motu Motu 2 (Untreated) , January, 1945											
Less than 2	133	17	6	5	0	2	2	F+V, 4	2	3.8	91
2-5	34	9	4	7	1	2	1		7	2.9	74
6-10	58	10	4	6	1				9	2.5	45
11-15	45	8	1	3	1				10	1.7	49
16 and over	131	18	4	3	3	3			16	1.6	32

In Motu Motu 1, ten children were born after spraying, three had palpable spleens, four had positive smears

In Motu Motu 2, one child was born—spleen not palpable Smear positive

\* T = Trophozoite G = Gametocyte V = vivax F = falciparum M = malariae

in nature Precipitin tests showed that 17 per cent of the seventy positive specimens had fed on humans It must then be considered a vector on the south coast of Papua

As a means of military control of malaria, the importance of spraying DDT inside native houses in villages near military installations is confirmed Since *Ap. moluccensis* has been found resting in tents, spraying the inside

of tents is also indicated, for most malaria transmission in troops in New Guinea came from the troops themselves despite the ordered use of atabrin for suppression.

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## DDT AIRSPRAY IN MALARIA CONTROL IN EAST AFRICA\*

BY

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### THE PROBLEMS

Of the species of predominant importance in the transmission of malaria in tropical Africa, *Anopheles funestus* is in our experience comparatively easy to control, for its breeding places are localized and may be rendered unsuitable to this species by larvicidal or other measures. *A. gambiae*, on the other hand, is ubiquitous in its breeding places, and these may change almost from day to day. As a result even the most careful larvicidal programme backed by necessary drainage may, in bad areas, leave a considerable residuum of this species, enough in fact to cause a high malarial rate. But this partial failure of control is confined in most places to the periods of high and continuous rainfall, when *gambiae* breeding may occur almost anywhere.

Secondly, these two species may breed prolifically in swampy areas which may be so large or inaccessible that high capital expenditure is required to make them amenable to ground larvicidal measures.

Thirdly, the range of flight of *funestus* is known to be of up to 4.5 miles (ADAMS, 1940), and of *gambiae* up to 3 miles (DE MEILLON, 1937). How these flight ranges may affect practical malaria control is undetermined. The radius of a military control at Dar-es-Salaam had been extended to nearly 2 miles, and it was uncertain how far the residual anopheline population was attributable to the missing of breeding places within this area, and how far to an insufficient radius of control.

\* This paper is published with the permission of the D D M S, East Africa Command. Acknowledgment is made of the assistance in practice and in many discussions of Flt/Lieut G A BECK, R A F, and to the officers and men of 1345 Flight, R A F, without whose interest and co-operation so much less would have been achieved. We are also much indebted to Mr V A BECKLEY, of the Scott Agricultural Laboratories, for chemical estimations involved, and for others to Captain R L HARTLES, R A M C.



Fourthly both species spend a large proportion of their life within houses, and it is improbable that an aerospray composed of large particles would reach the adults of these species. On the other hand it might be expected that an anti-adult measure such as house impregnation would be the most profitable line of attack but regular and efficient impregnation of a large urban area is not a practicable undertaking.

The aerospraying here recorded was planned in relation to the foregoing four main problems inevitably other questions obtruded themselves from time to time, and these are to some extent discussed.

The general effectiveness of DDT aerospray as a larvicide against other mosquito species was already established before the present observations were undertaken, but the considerations summarized above seemed to offer some applications of aerospray of particular value to conditions in tropical Africa.

Firstly a blanket treatment, of a whole area liable to provide the many breeding places of *A. gambiae* during the rains, might be expected to overcome the great difficulties experienced in obtaining a complete coverage. Again, there could be no doubt that the problem of inaccessibility should easily be overcome by attack from the air. The question of the radius necessary for effective control should equally easily be answered by the use of this method provided aerospray were effective with a minimum of ground preparation. It was not expected that much light would be thrown on the relative merits of larval control by aerospray or adult killing by house impregnation.

#### PLAN OF TRIALS.

Two sets of observations, including three series of sprayings, are recorded, both of which refer to aerospray programmes which were intended to be practical anti-malarial measures. An endeavour has been made to assess these sprayings as accurately as possible, by their entomological results unfortunately they could not be assessed in terms of malaria incidence, as the Service populations at risk were taking suppressive mepracrine and this had not been the case in previous years. From time to time modifications of procedure were made in order to elucidate particular problems that arose in the course of the trials, and at the end of the control period a number of purely experimental sorties were flown.

The first series of observations was made at Dar-es-Salaam Tanganyika, between 15th April and 25th May 1945. The central area of control, about 2½ miles in radius, was given six weekly sprayings. This series was intended to reveal the improvement on ground control that might be effected by superimposing aerospray at an assumed dosage of 17 mg. per square metre of pure DDT. The second series was carried out also at Dar-es-Salaam between 27th May and 23rd June consisted of four weekly sprayings over an outer zone of 2 to 4 miles radius from the centre omitting most of the central zone previously sprayed. The dosage was the same as before, but this series was intended to

reveal whether a greater radius of control would further decrease the anopheline population at the centre. The third series was carried out at Kisumu, Kenya, between 14th July and 15th September, and consisted of five weekly sprayings, followed by two sprayings at intervals of a fortnight intended to show what loss of efficiency was caused by this longer interval between sprayings. The general object of this series was to observe the effect of an increased dosage of 32 mg per square metre of pure DDT.

Throughout this paper DDT dosage is given as pure DDT in milligrammes per square metre. For the convenience of readers a table of the rates of application used, in various terms, is given below.

TABLE I  
AIRSPRAY DOSAGES USED

Spray mixture		Crude DDT (59-67 per cent) Oz per acre	Pure DDT Mg per square metre	Concentration (pp DDT) per cent.
Imperial quarts per acre	Imperial gallons per square mile			
1.5	240	3.9	17	4
2.25	360	7.1	32	5

#### MATERIALS AND METHODS IN SPRAYING

The DDT used was of a stated 59 to 67 per cent *pp* content, and the dosages given of "pure" DDT are based on the mean of this range of purity. The DDT was dissolved by heating on water-baths built out of 44-gallon drums, in a proportion of the diesolene (A G O) which was then added to a mixture of the remainder of the diesolene and furnace oil, giving a final mixture of 50/50 of these two oils. The choice of materials was determined by availability, combined with certain criteria of viscosity and specific gravity, in order to obtain as close an identity as possible with a mixture of equal parts of diesolene and lubricating oil (H D 50). Such a mixture had been used in earlier trials in Mid-East, and the height/wind tables first used were based on its properties.

The spreading pressure of the mixture was low during the first series of trials (less than 16 dynes), but for the second series some oleic acid was obtained, and the addition of 0.5 per cent of this increased the spreading pressure to 20 dynes. In the third series a locally prepared fatty acid residue was added at the rate of 0.5 per cent, and this addition gave a spreading pressure of 16 to 20 dynes or more.

The gross weekly expenditure of spray mixtures corresponded closely to the calculated expenditure based on assumed dosage.

The oil mixture was carried in a 300 gallon bomb-bay tank and was emitted through a single 2.5 inch emission pipe fitted with a circular venturi flume. The emission rate could not be varied, and was about 2 gallons per second.

Spraying height was at first based on windspeed and direction, measured on the aerodrome from which flying was taking place, but at Kisumu much more reliance was placed on the indications given by generators at the site of spraying. These smoke generators were always used to indicate the actual spraying track to be followed. In the first series at two points 2 miles apart along the track, and in the other two series at the beginning of the track the pilot in that case flying on a fixed bearing. The points at which generators were ignited were determined by actual measurement on the ground, and so far as possible roads or railways were chosen for the base lines. Although the

pilots had in every case a previous long experience of flying the Baltimore aircraft employed, the efficiency and accuracy of spray application undoubtedly improved with the increasing experience of spraying technique. This improvement was in part due also to the parallel familiarization with the method of the personnel on the ground.

The height at which spraying was carried out is most simply conveyed by examples of the actual height of flying at two typical windspeeds, the wind being in each case directly on the beam. Thus in Series I and II the height was 310 feet with cross wind of 8 m.p.h., and 610 feet with wind of 4 m.p.h. In Series III when the wind was 8 m.p.h. the height was about 200 feet, and 400 feet when the windspeed was 4 m.p.h. On account of the prevailing winds encountered, the flying height was most commonly in the region of 350 to 600 feet in Series I and II and 200 to 350 feet in Series III.

#### METEOROLOGY

The Series I sprayings commenced some 2 weeks ahead of the normal period of maximal rainfall, and thus well before the annual peak of anopheline incidence at Dar-es-Salaam would be expected. The rainfall actually reached its peak during the fourth of the six weekly sprayings, and it was adjudged that the numbers of anopheline vectors in untreated areas reached their maximum at about the same time. By the commencement of the Series II sprayings both rainfall and *Anopheles* were decreasing—in fact, the annual season of heavy rain was virtually over.

The Series III sprayings did not commence until the rainy season (less well defined at Kisumu) was well advanced, and anopheline incidence was just reaching its peak for the season.

Rainfall figures for the two locations given in Table II show that total rainfall during the spraying periods was rather less than the average for the previous 4 years at Dar-es-Salaam, whereas at Kisumu it was considerably heavier than the average.

TABLE II  
RAINFALL

	Dar-es-Salaam.			Kisumu.		
	4-year monthly average.	Monthly totals. 1948.	Days of rain. 1948.	4-year monthly average	Monthly totals. 1948.	Days of rain. 1948.
Jan.	2.49	3.40	18	1.63	3.14	18
Feb.	3.73	2.19	9	3.78	1.93	7
Mar.	4.00	5.85	18	6.45	1.97	4
Apr.	12.38	7.29	20	6.74	3.67	10
May	7.71	8.63	22	7.39	6.33	24
June	0.92	20.40	4	4.21	6.77	16
July	1.29	0.4	5	1.84	1.68	10
Aug.	1.10	0.75	6	2.60	7.21	13
Sept.	1.10	1.75	10	.42	20.63	4
Oct.	1.37	4.04	10	1.73	.63	8
Nov.	2.19	.37	11	4.64	1.39	11
Dec.	3.74	1.44	9	3.66	1.84	3
	43.89	28.81	158	47.37	41.31	119

\*spraying period.

The all-important weather conditions for spraying technique are windspeed and direction. At Dar-es-Salaam southerly winds were remarkably constant, particularly between 8.0 a.m. and noon, and spraying was therefore confined to the mornings. At Kisumu wind direction was much more variable, particularly in the mornings, but between 2.0 and 6.0 p.m. the direction was reasonably constant and from the south-west, and spraying was accordingly devised so that as many sorties as possible should be flown from north-west or south-east during the afternoons. Comparable surface windspeed figures for the two places are given in Table III. It will be seen that at Kisumu light winds were much less frequent in the afternoons than in the mornings, so that windspeed, as well as wind direction, was more favourable at that time.

TABLE III  
SURFACE WINDSPEED FREQUENCIES

Windspeed	Dar-es-Salaam		Kisumu	
	9.0 a.m.	2.0 p.m.	9.0 a.m.	2.0 p.m.
1-3 m.p.h.	15	14	15	1
4-7 "	39	22	29	22
8-12 "	15	27	5	23
13-18 "	1	7	0	3

Other meteorological conditions during the air-spraying periods are briefly summarized in Table IV. The features to be noted are the progressive reduction in minimum temperature throughout the period of Series I and II, and the falling humidity as the rains decreased. Conditions in the two places were fairly similar, with the exception of afternoon humidity, which was noticeably lower at Kisumu, and of the minimum temperature which was on an average 9° lower, and at the same time less variable, than at Dar-es-Salaam (Table IV, page 828).

#### ENTOMOLOGICAL SAMPLING METHODS

Owing to the Service personnel at risk being given suppressive mepacrine, the means of assessment of the effectiveness of these sprayings was entirely entomological. A considerable number of catching stations was used, some of which were of long standing and others chosen at the time of the observations.

#### ADULT CATCHING STATIONS

Three categories were used in Series I and II, with a fourth in Series III. They were as follows—

##### *Series I*

*Eleven Central Stations*, wholly within the sprayed zone, and not less than 1 mile from its boundary, except where this abutted on the adjacent municipal control.

*Fifteen Marginal Stations* along the perimeter, but within the sprayed zone.

*Nine Extra Limital Stations*, not less than 500 yards outside the boundary of the sprayed zone.

##### *Series II*

*Eleven Central Stations* as for Series I, but now in the unsprayed central zone.

*Twenty-four Marginal Stations* now including the original extra limital stations.

*Six Extra Limital Stations* newly selected to be at least 500 yards outside the boundary of the wider sprayed area.

## Series III

*Eight Central Stations* in the unsprayed central zone.

*Six Marginal Stations* in the sprayed area, but at least 800 yards from its outer Margin.

*Five Extra Limited Stations* outside the spraying perimeter

*Five Airspray Stations* more than miles within the outer limit of the sprayed zone, and yet not appreciably affected by the ground control. Any changes in these stations was thus believed to be wholly due to the airspray

TABLE IV  
METEOROLOGICAL DATA.

Fortnight ending	Mean minimum.	Mean maximum.	R.H. 0830 L.	R.H. 1430 L.	Rainfall
Dar-es-Salaam.					
14.4	73.2	83.7	80	78	8.48
20.4	73.3	83.6	80	83	1.17
12.6	72.2	83.7	81	83	7.73
20.6	72.0	84.7	87	72	1.62
8.6	80.7	85.1	84	88	0.07
22.6	83.8	83.6	78	81	0.86
Mean	76.7	83.7	86	83	3.87
Average maximum	74.7	82.2	83	83	(3.31)
minimum	80.3	79.0	80	80	(—)
Kisumu.					
14.7	82.8	80.2	81	83	1.13
22.7	84.1	80.5	80	80	0.38
11.8	83.8	79.2	81	83	4.91
23.8	84.6	81.6	87	80	0.32
8.8	81.7	81.2	86	80	2.14
22.8	80.2	84.7	83	44	0.28
Mean	81.8	81.2	87	87	1.43
Average maximum	80.7	87.6	85	88	(1.84)
minimum	84.4	72.1	72	33	(—)

Each adult station was composed of group of African huts, usually three in number, at Dar-es-Salaam, and four or five at Kisumu. With the exception of five municipal stations at Kisumu (in which larger number of rooms was searched for much briefer period by municipal searchers), searching was carried out by African malaria assistants under the supervision of R.A.M.C. officers or N.C.O.s hand catching with test tubes was used. Catching time was not less than 20 minutes, and was extended beyond this according to the numbers of mosquitoes to be caught, the objective being to catch all visible mosquitoes.

TABLE V  
MEAN LARVAL CATCHES PER 100 DIPS (GEOMETRIC MEANS)

	Week ending	Sprayed areas Larvae				Unsprayed areas Larvae			
		Anoph- eles III and IV	All Anoph- eles	All Culex	All larvae and pupae	Anoph- eles III and IV	All Anoph- eles	All Culex	All larvae and pupae
Before spraying	14 Apr	Series I—Dar-es-Salaam				Dosage 17 mg			
		—	226	146	476	—	162	24	242
	21 "	—	48	30	92	—	124	56	254
	28 "	—	86	30	108	—	172	50	284
	5 May	—	22	28	60	—	172	98	318
	12 "	1.0	4	4	8	8.0	68	12	94
	19 "	0.3	4	6	10	4.2	122	24	192
Spraying period	26 "	0.5	28	10	72	0.6	108	28	160
After spraying	2 June	Series II—Dar-es-Salaam				Dosage 17 mg			
		0	4	12	22	15.6	58	54	226
	9 "	0.4	8	12	20	7.4	190	108	348
	16 "	1.0	32	56	108	12.8	174	166	388
	23 "	0.2	6	20	34	3.0	64	44	110
	30 "	1.2	20	70	80	1.0	62	54	136
	7 July	2.2	58	110	210	10.8	218	34	324
After spraying	14 "	2.6	144	102	266	9.2	254	86	404
Before spraying	4 July	Series III—Kisumu				Dosage 32 mg			
		—	150	71	287	33.7	154	39	212
	11 "	18	147	106	267	36.2	161	160	223
	18 "	0	13	22	81	1.5	31	223	340
	25 "	0	0	40	58	9.7	46	164	173
	1 Aug	0	10	17	20	2.8	59	177	281
	8 "	0	0	14	14	4.6	34	1	61
Spraying period	15 "	0	0	11	11	6.9	50	23	73
	22 "	0.1	15	71	75	12.2	52	88	127
	29 "	0	16	17	15	1	17	7	104
	5 Aug	0	1	76	77	0	2	41	55
	12 "	0	0	17	17	7.9	49	75	169
	19 "	—	—	—	—	4.2	4	—	77
	26 "	—	68	11	79	1.7	2	15	41
After spraying	2 Sept	—	17	17	17	1.7	7	—	16
	9 "	—	17	17	17	—	16	2	18

## LARVAL CATCHING STATIONS.

Larval sampling consisted in making a set number of dips per station, with ladles. The larvae were counted and classified by stages and genera. In Series I and II fifty dips were made and counting was by naked eye, the larvae being returned to the water. In Series III, 100 dips were made, and the larvae taken to the laboratory for classification and identification of the older stages. Dipping was carried out twice weekly at Dar-es-Salaam, the day before and 2 days after the weekly spraying, and was confined to certain marked patches in the breeding areas chosen. At Kisumu dipping was carried out once weekly, and at random in likely breeding water over an area of approximately 500 square yards for each station. The number of larval catching stations in the sprayed areas was six in Series I eight in Series II, and eight in Series III while in the unsprayed areas there were four five and three respectively. In the treated larval stations ground control was discontinued for the period of the spraying.

## ENTOMOLOGICAL RESULTS.

The high order of natural larval mortality made the assessment of larval changes more difficult than had been anticipated more particularly in the case of the *Anopheles*. The mean weekly percentage of Stage III and IV larvae were as follows

	Anopheline	Culicine
Dar-es-Salaam	7	17
Kisumu	18	24

The lower dosage used in Series I and II, of 1 mg per square metre gave an initial 80 per cent larval kill of all stages and no consistent decrease resulted from further sprayings. At this dosage moreover the mean weekly reduction of post-spray on pre-spray catches was only about 0 per cent in spite of the substantial rises in number of larvae that almost invariably occurred in the intervals between spraying. A very much higher order of kill was, however achieved in Series III at a dosage of 32 mg per square metre. In this series an initial mean catch of 280 was reduced to eight per 100 dips after the first spraying a reduction of 97 per cent. This initial reduction was succeeded by some further decrease even when allowance is made for the seasonal reduction shown by the unsprayed stations. No systematic weekly pre-spray catches were carried out during Series III, but some catches that were made in the intervals showed no rise in larval numbers comparable to those that occurred in the other series. When the spraying interval was increased to two weeks (29th August and 2nd September) larval numbers between sprayings did not rise above 7 per cent of those in the unsprayed area.

Larval control during Series I and II was thus far from complete, whereas in Series III it was very nearly complete. At the higher dosage there was a suggestion of some cumulative effect of the DDT treatment but only slight if any evidence of such an effect at the lower dosage.

Mean weekly catches of adult vectors are shown in Table VII and Figs 3 and 4 and are summarized in Table VIII. These show an even greater contrast between Series I and II on the one hand and Series III on the other.

than do the larval catches, but it must be emphasized that seasonal changes in anopheline prevalence add to the apparent contrast to an extent that is not the case in the larval catches.

TABLE VI  
SUMMARY OF MEAN LARVAL CATCHES PER 100 DIPS

Spraying	Sprayed area.		Unsprayed area	
	<i>Anopheles</i> III and IV	All larvae and pupae	<i>Anopheles</i> III and IV	All larvae and pupae
<i>Series I</i>				
Before spraying	—	476	—	242
Spraying period	0.8	55	4.3	217
<i>Series II</i>				
Spraying period	0.4	47	9.7	268
After spraying	2.0	187	7.0	288
<i>Series III</i>				
Before spraying	22.0	277	34.9	242
Spraying period	0.1	4	5.3	154
After spraying	0.3	2	2.1	36

There was, however, an immediate fall in the adult catches following the first spraying in Series III, whereas in both Series I and II a reduction only occurred later and to a less extent than in the former case. There was in fact an increase both absolutely and relative to the Extra Limital stations, in the Central Stations during Series I. This increase did not occur to any appreciable extent in either the Marginal or Extra Limital Stations, and it must be assumed that breeding was to some extent uncontrolled in the central zone. There was not, however, the seasonal upsurge in the Central Stations that had occurred in previous years, as is indicated in Table IX; other results for previous years are not shown as, owing to differences in rainfall and the execution of certain major works, it is not considered justifiable to draw any more definite conclusions from them than those given above. Anopheline catches remained at half those of 1944 for 3 months after the completion of spraying.

The effect of the Series II spraying is equally indefinite, at least in the central area. In the Marginal Stations there was some evidence of an effect from airspray (in these there was no question of a contributory effect from ground control) after 3 weeks spraying, an impression reinforced by the apparent increase after the cessation of airspraying. But there was no greater increase in the Marginal than in the Extra Limital catches. What little effect there may have been seems to have been chiefly on *A. funestus*. Owing to the absence of previous ground control the immediate reduction resulted



TABLE VII  
MEAN ADULT VECTOR CATCHES PER NET SEARCHED.

Week ending	Alopray		Control.		Marginal.		Extra-Marginal.	
	All vec. tons.	Per cent. fowles.	All vec. tons.	Per cent. fowles.	All vec. tons.	Per cent. fowles.	All vec. tons.	Per cent. fowles.
Before spraying	31 Mar	—	Series I—Dar-es-Salaam. Doseage 17 mg.		—	—	—	—
	7 Apr	—	0.29	25	5.8	49	—	—
	14	—	0.54	21	5.4	29	—	—
	21	—	0.22	21	7.0	23	18.4	47
Spraying period	28	—	0.77	39	9.7	36	20.1	33
	5 May	—	2.71	10	8.4	31	18.6	29
	12	—	0.93	31	6.1	23	14.1	29
	19	—	0.23	37	8.9	37	19.1	41
	26	—	0.92	0	9.6	27	23.2	24
	2 June	—	1.62	4	10.8	34	18.6	32
After spraying	2 June	—	1.16	7	6.2	23	18.7	30
	9 June	—	Series II—Dar-es-Salaam. Doseage 17 mg.		—	—	—	—
	16	—	1.18	16	8.2	23	19.2	24
	23	—	1.02	12	8.0	28	8.2	24
After spraying	30	—	0.76	23	1.6	19	8.7	30
	7 July	—	0.22	6	1.1	28	5.5	31
	14	—	0.18	0	1.1	27	3.7	36
	21	—	0.27	30	2.6	31	4.8	40
Before spraying	23 June	—	Series III—Kumru. Doseage 22 mg.		—	—	—	—
	30	—	4.68	12	—	—	20.4	11
	7 July	—	3.28	7	28.1	21	21.2	10
	14	—	2.71	24	25.0	87	29.6	10
Spraying period	21	0.89	26	0.92	8	4.8	84	27.0
	28	0.29	24	0.48	8	1.9	88	17
	4 Aug.	0.60	11	0.37	6	1.2	41	11.8
	11	0.18	56	0.29	13	0.2	34	7.7
	18	0.06	84	0.12	0	0.3	21	6.7
	25	0.30	10	0.01	6	0.2	21	3.7
	1 Sept	0.34	16	0.03	0	0.4	28	5.2
	8	0.02	6	0.01	6	0.8	48	3.2
	15	0.19	63	0.02	1	0.8	48	1
	22	0.21	100	0.01	0	0.4	29	3.4
After spraying	29	0.02	100	0.02	6	0.8	78	1.7
	6 Oct	0.06	100	0	—	0.3	48	0.8
	13	0.18	63	0.07	28	0.4	61	0.8
	20	68	87	0.06	32	0.4	60	0.8

Note.—Sprayings indicated by horizontal lines.

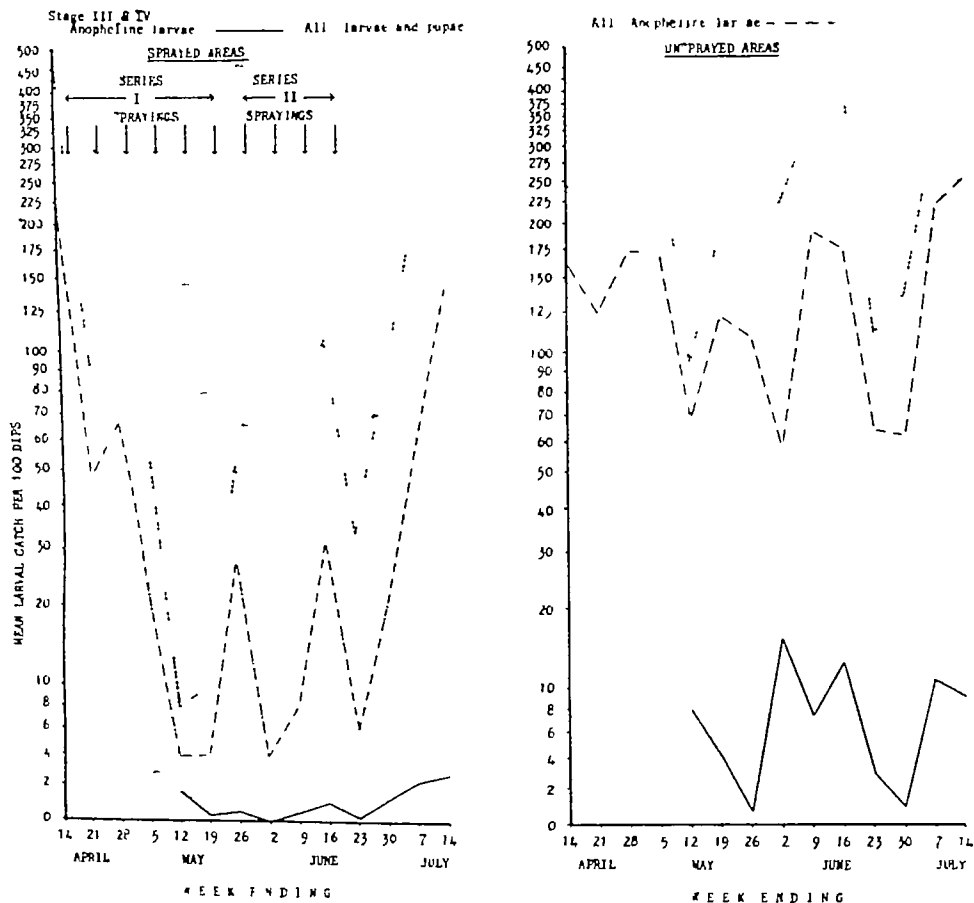
FIG 1 LARVAL CATCHES PER 100 DIPS  
(Geometric means)

TABLE VIII

MEAN ADULT VECTOR CATCHES EXPRESSED AS PERCENTAGES OF EXTRA-LIMITAL CATCHES

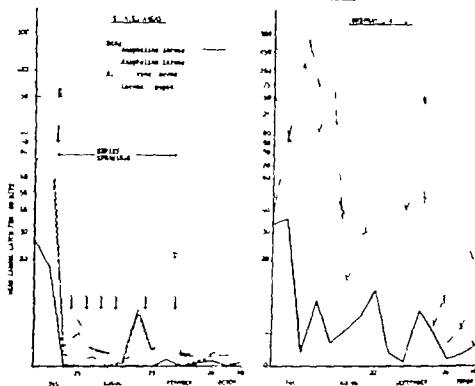
		Airspray	Central	Marginal
<i>Series I</i>	Before spraying	—	3	47
	First week	—	17	52
	Spraying period	—	8	49
<i>Series II</i>	Spraying period	—	7	42
	After spraying	—	4	44
<i>Series III</i>	Before spraying	131	11	64
	First week	2	4	19
	Spraying period	4	3	16
	After spraying	24	3	39

TABLE IX.  
CORRESPONDING CENTRAL STATION CATCHES FOR THREE YEARS  
AT THE SPRAYING PERIOD WEEKLY MEANS.

	1942	1944	1945
Dar-es-Salaam	2.43	4.30	1.06
Kisumu—			
All central stations	—	0.76	0.18
Municipal stations only	0.16	0.08	0.04

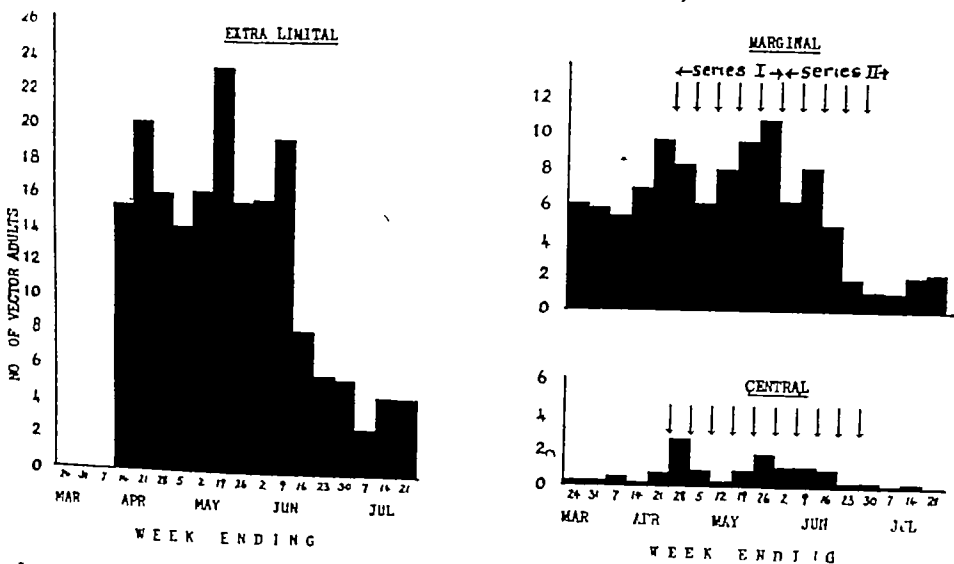
FIG. 2. LARVAL CATCHES PER 100 DIPS  
(Geometric means).

LARVAL CATCHES PER 100 DIPS (Geometric means).



from the first coverage in Series III was greatest in the Airspray Stations, the reduction in catches in these stations being 98 per cent and relative to the Extra Larval catches even greater than this. But there was a coincident

FIG 3 ADULT VECTORS PER HUT  
(Sprayings indicated by vertical arrows)



decrease in the catches in Central Stations of 66 per cent, and in the Marginal Stations of 81 per cent. As was to be expected the numbers in the Marginal Stations did not fall to as low a figure as in the two former groups. When spraying ceased there was an increase in all stations, the catches in both Marginal and Airspray Stations returning on 20th October (see Table VII) to a figure that approximated to the Extra Limal. Apparently, however, this recovery in anopheline prevalence was not enough to lead to a re-invasion of the central controlled area. Some further evidence in this direction is provided by later catches in the municipal area, for during the succeeding 6 months anopheline catches were less than 1 per cent of the average of the preceding 3 years. The effect appears to have been greatest against *A. gambiae*. Although there is normally a succession of *gambiae* by *funestus* at Kisumu at this time of year (GARNHAM, 1938), there is a noticeable difference between the proportions of these two species in the different groups of catching stations, and re-invasion of the treated areas was mainly by *funestus*. The seasonal increase in *gambiae* seems to have been checked.

#### MODE OF ACTION OF AIRSPRAY USED

The intended point of attack against *Anopheles* in the sprayings here discussed was against larvae, and larvae were killed with a completeness that varied with the dosage. In the third series however the rapid fall in adult catches carries a strong suggestion of an anti-adult action in addition. Reports from America, for example KNIPPLING (1945), have laid stress on such anti-adult action against other mosquito species but the effect seems chiefly to

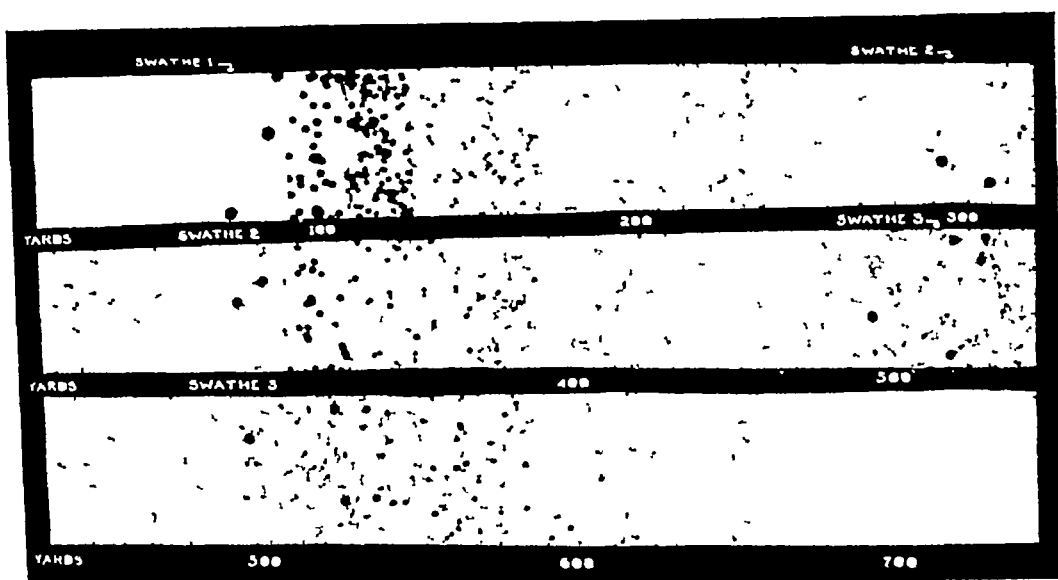
such cover it is necessary to make allowance for the vicissitudes of windspeed and direction. The variation in these factors will inevitably lead unless a correction be made to patches of underdosage although a very constant wind may reduce the need for correction to such an extent that it may be ignored.

Variation in ground dosage attributable to changes in wind conditions received more attention at Kijumu, where this variation was particularly evident. It was not possible to make regular estimation throughout the spraying so that sufficient data to reveal the extent and frequency of such variations could not be obtained. But some notion of their range was given by the estimations carried out and shown in Table XI. These represent the results of chemical estimations of series of papers put down at interval across two or three overlapping swathes, flown at the usual 200 yard intervals. Similar variation was more often observed visually on the test papers regularly laid down. It will be seen that the maximum and minimum dosages actually sampled chemically were 74 mg and 14 mg/sq metre respectively. Allowing for all experimental error (which must necessarily be great), it is evident also that the differences in a single swathe are commonly as great as those between different swathes. On the other hand steady wind conditions were necessarily chosen for such special tests.

Variations in ground dosage at any given distance from the spray track of the aircraft frequently arise from the variability of the wind. This variability may be divided into two categories of which the first is wind turbulence proper. This causes only limited local effect and is unpredictable except in so far as conditions generally favourable for the production of convection current may be foreseen in practice it may render praying quite ineffective. The second category is that of changes in windspeed and direction these profoundly affect not only the distance downwind at which the swathe will start to reach the ground but also its width: they can be allowed for according to the accuracy with which wind measurement can, under the circumstances, be made. The effect of unpredicted wind changes can be seen in Table VI by the varying point of commencement of Swathe II. But there is a further variation in each swathe that is more constant than the variations due to wind condition. It is more clearly shown in Table VI and is apparently inseparable from the practical distribution of an aircraft. Dispersion of the spray which is illustrated in Fig. 5 is dependent upon the differing rates of fall of varying sized droplets, and the dosage given by the small number of large drops falling at say 100 yard from the aircraft track can seldom if ever be the same as that given by the larger number of smaller drops falling 100 yard further downwind. On observation of the dosage pattern across the swathe used, however a rise across the first 50 yards to a maximum in the second 50 yards and a gradual decline in the last 100 yards.

The height/wind product of course determines the width of the swathe and given fixed interval between swathes, the amount of overlap from

FIG. 5. DROPLET DISTRIBUTION OF AIRSPRAY ACROSS THREE SUCCESSIVE SWATHES



swathe to swathe. All the pairs of swathes to which Table XI refers were flown at intervals of 200 yards. It was found that an overlap of at least 100 yards was obtained in most cases, and observations on the test papers have shown some spray falling over 360 yards downwind from the upwind edge of the swathe.

This important question of height and windspeed is outside the province of the present writers, as in fact is most of this section of our discussion, but it has been necessary to enter into these matters as they are integral to the all important questions of dosage.

#### EFFECT OF SHADE ON THE PENETRATION OF SPRAY

There are two ways in which vegetation may interfere with the larvicidal action of aerospray. First by preventing the spray from reaching the water surface, and second by preventing the comparatively small surface dosage of oil from spreading to all parts of the breeding area.

The second of these factors seems to be of comparatively small practical importance. The addition of oleic acid to the spray mixture during the second series of sprayings did not effect any apparent improvement in larval kill (see the period 2nd to 23rd June, Fig. 1, on page 833). Moreover, a series of trials by hand spraying, on pools with a moderate growth of grass up to 1 foot in height failed to show any improvement when mixed fatty acids (obtained from the



FIG. 6. TYPE I SHADE, OBLIQUE VIEW  
Grass 12 to 18 inches high.



FIG. 7. TYPE II SHADE, OBLIQUE VIEW  
Grass 12 to 30 inches high.



FIG. 8. TYPE I SHADE, VERTICAL VIEW



FIG. 9. TYPE II SHADE, VERTICAL VIEW



FIG 10 TYPE III SHADE, GENERAL VIEW  
Sedges and papyrus of varying height up to 8 to 10 feet

The practical implication of these findings is that more than double the amount of DDT necessary on the water surface may have to be distributed from the air. A commonly accepted minimum dosage of pure DDT per square metre is 10 mg, and the dosage distributed in our first two series of trials was 17 mg. One possible explanation is thus provided for the relative ineffectiveness of larvicidal action in these trials.

A further conclusion that may be drawn is that it should be possible, and is desirable, to regulate the rate of emission to the prevailing vegetational type found in the area over which airspraying is being undertaken.

#### DOSAGE

There can be no doubt that the first of the two dosages used was too low, and it has seemed to us probable that the second was higher than necessary. It is unfortunate that, in order to increase the DDT dosage to the extent desired, it was unavoidable that the amount of oil carrier should also be increased. It is accordingly impossible to do more than express an opinion as to the relative importance of DDT alone, or of total aerosol mixture, in accounting for the differences observed.



TABLE XIII.

PENETRATION OF AIRSPRAY THROUGH VEGETATIONAL COVER OF SEEDING GROUNDS.

Density	Number of drops.			Amount of DDT in mg.		
	Not shaded.	Shaded.	Per cent. penetration.	Not shaded.	Shaded.	Per cent. penetration.
I	245	161	47			
	776	423	56			
	51	30	50			
	1,303	671	67			
	666	451	68			
	1,325	699	49	57	37	65
			—	41	42	100
		Mean	54	70	64	91
				79	45	57
II	712	142	20			
	1,841	653	44			
	2,021	840	47			
	1,543	280	25			
	908	287	30			
	2,300	683	28			
	410	117	29			
	3,345	1,049	32			
	291	493	65			
		Mean	34	47	33	73
				76	61	81
				89	19	21
III	1,075	284	4			
	825	288	25			
	753	116	16			
	2,018	218	11			
	900	262	29			
	1,385	831	35	57	23	40
		Mean	23	76	34	45
				48	12	27
Potia	~896	1,067	37			

The considerations already discussed, under the headings of penetration and characteristics of the swathe, are those that must determine the adequacy of dosage over and above the minimum required on open water and under ideal conditions. It remains to relate these considerations to actual quantities required in the field.

On account of the density of vegetation that is likely to occur in and around typical *A. gambiae* breeding places, the minimal dosage of DDT necessary to effect a complete kill may have to be doubled, and in *funestus* breeding places more than doubled, to allow for the "wastage" of the spray that occurs on the vegetational cover of these breeding places. Assuming that 10 mg per square metre of pure DDT is required in open water, then at least 20 to 25 mg per square metre is necessary for average grass cover, and even more than this when breeding places are likely to be sheltered by a heavy sedge cover.

The minor variations in spray distribution that are due to wind turbulence may have to be accepted as an unavoidable limitation on the method, but the variation that is found across the width of a normal swathe must be considered in determining dosage. If the downwind half of a swathe be accepted as the measure of what may be counted upon to reach the ground, then it is apparent from Table XI that the average amount released should be 40 to 50 per cent greater than the required minimum dosage at every point. It is apparent that the more swathes can be overlapped the more fully can this type of variation be corrected.

Taking all these considerations together, it seems that, for the conditions under which the present sprayings were carried out, a dosage of the order of at least 25 to 30 mg per square metre is required. This conclusion is supported by what occurred during the actual course of the trials.

It is to be noted that the observed differences, on which this conclusion is based, are in quantities of DDT and not of total spray mixture. We have not been able to make an opportunity of correlating any failure of larvicidal action with deficiencies either of DDT or of spray mixture alone, and the practical requirement should quite possibly be expressed in terms of total spray rather than of its DDT content. On the other hand some later series of sprayings, not here recorded, at the same dosage of spray mixture as in Series III but with a lower concentration of DDT, giving 22 mg DDT per square metre, quite failed to show the dramatic results observed at Kisumu. Owing to the vagaries of wind direction and turbulence, great difficulties were experienced in obtaining even coverage during these sprayings, but a justifiable interim conclusion that may be drawn from this experience seems to be that a practical minimum amount of DDT that should be put down is more than 22 mg per square metre.

#### RADIUS OF APPLICATION

The area covered by both ground and air control at Dar es-Salaam was greater than is commonly supposed to be necessary for an effective anopheline control. On one side it was flanked by the municipal control area, and in all other directions it extended to a radius of 2 miles, in some parts to 2½ miles from the protected camp.

In spite of this control there remained in 1943 and 1944 in the innermost zone of 1 mile radius a mean adult house catch of three to four vectors per house at the peak period. This figure does not of course amount to more than about half of the total vectors in a house as it was obtained by hand catching.

From various considerations, in particular that of the catches in the Marginal Stations throughout the whole period we are of the opinion that the result of the airspraying was equivalent to an additional weekly oiling application by hand. The result was to reduce the catches in the innermost zone to a mean of less than one (0.72) per house during the peak of 1945. Although 1945 was as a whole a less favourable year for anopheline breeding than the previous year we conclude that breeding places had been and were still at the time of the airspraying, missed by the ground control. To this extent the observations give support to the notion that airspray may be a better method than ground control in dealing with *A. gambiae* in open country.

Reference to Tables VII and VIII and Fig. 3 shows that no improvement on these inner zone catches was effected by the extension of the radius of air control to nearly 4 miles, for the ratios between Central and Extra-Limital catches during the two series of sprayings were respectively 7.8 and 7.2. This gives further support to the conclusion that some breeding was being missed by the ground control. Even though the larvicidal effect of the air spray was incomplete, it further reduced anopheline infestation when the inner zone was covered but it did not reduce it appreciably further when many more larvae over a much wider area were being killed. It seems justifiable to conclude therefore, that the reason for ineffectiveness in the control of these two species is to be sought rather in the missing of breeding within the control than in the inadequacy of the area covered, if this extends to a 2 mile radius.

Positive evidence for this last conclusion is given by the third series of sprayings. The villages represented by the airspray catching stations were wholly surrounded by a zone of airspray. This zone was nearly 1½ miles wide in all directions except that of the open Lake Victoria. The initial 98 per cent reduction in adults which was fully maintained over a period, seems to give a clear enough indication that this radius of control, when larval kill was complete gave an efficiency as great as that of the much wider radius of control at Dar-es-Salaam.

Reference to Table VIII shows that the ratio of Airspray to Extra-Limital catches was 3 per cent. in the third series, as compared with 7 per cent. between the Central and Extra-Limital catches in the second series, but there still remained an average anopheline catch of 0.3 per hut in the airspray zone. The numbers actually caught probably represented a relatively greater actual house population at Krumu than on the coast chiefly on account of the different type of house construction, and the consequent greater difficulty in hand catching mosquitoes. In spite of the evident high efficiency achieved

rapidly and simply by this method of control, there was still therefore no complete elimination of *Anopheles* even at Kisumu

With all due care to avoid unjustifiable dogmatism, it is possible on the basis of the experiences in these two widely differing places to form some conclusion as to the necessary radius for practical control of these two species of African *Anopheles*. The minimum radius should be between  $1\frac{1}{2}$  and 2 miles if the maximum effect is to be achieved. It does not appear to be necessary to extend control further than this.

#### EFFECT ON CROPS AND FISH

It was not possible, during the course of these trials, to make any detailed observations on the effect of the spray on crops, and at Kisumu the millet crop had already set before spraying commenced. Considerable areas of rice were sprayed at Dar-es-Salaam, but no harmful effect was observed here, nor were any such effects commented upon by the cultivators.

Some much fuller observations were made on fish mortality at Kisumu by Dr V D VAN SOMEREN. These may be summarized as follows. Although no effect on fish, and only a slight effect on plankton, could be found following a single spraying at a dosage of 32 mg per square metre, there was an undoubted mortality among inshore fish in a limited area, following seven sprayings. The area affected was on the lee shore of the lake, and it must be assumed that an accumulation of spray was likely to occur here. There was, in addition to the mortality in fish (mainly *Haplochromis* sp and *Tilapia variabilis*), a great reduction in most aquatic insects. In a swamp area covered by these sprayings, there was no apparent fish mortality, but there was a reduction of some groups of insects, particularly dipterous fly larvae and mayfly nymphs. In both areas no apparent effect was observed on snails and leeches, and dragonfly nymphs appeared to be little affected. It was concluded that although fish might be killed at this dosage, by some means as yet undetermined, it was unlikely that spraying over a limited area of open lake would have any appreciable effect on fisheries as a whole.

#### GENERAL CONCLUSIONS

There are many gaps in the foregoing sets of observations, and it may well be considered that any assessment based on them, of this comparatively new method, is both premature and unjustified. Since, however, it is a method which opens up a number of new possibilities in the control of malaria, some such assessment in relation to African *Anopheles* has seemed to us to be desirable now, even if in the upshot some of the conclusions drawn prove to be erroneous.

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1 The discovery of the insecticidal value of DDT has made possible for the first time the application in comparatively small quantity of a mosquito larvicide, moreover the oil carrier in which it may be distributed is comparatively easily controllable. In turn airspraying makes the control of inaccessible breeding places possible though it does not at the present price of DDT appear to be an economic alternative to larvicidal ground control when that is possible and equally effective.

2 Inaccessibility to ground control may arise from two causes either the existence of some impassable barrier usually water or the breadth of breeding area to be covered. This latter obstacle may compel large drainage schemes as a necessary preliminary to efficient spraying from the ground. Of the two causes the extent of breeding areas is more likely to lead to economic applications of airspray for there is little doubt that under most circumstances, this method offers an alternative to large capital works that is both cheaper and at the same time more efficient.

3. A further advantage of airspray which may have application in peace as in war is the speed with which control may be established, with no more preliminary survey than a rapid reconnaissance from the air. The value which this might have in the face of an epidemic is very evident.

4 The possible disadvantages of the widespread distribution of DDT remain to be explored but, while recognizing the potential danger it seems to us that this is at present being exaggerated. There is frequently some economic loss arising from the application of anti-malarial measures, and it is always necessary to balance the losses likely to be incurred because of anti-malarial measures against the losses attributable to the malaria which they are designed to prevent. Any general upset over a wide area, of a biological equilibrium between insects of economic value and those which are noxious seem to be improbable in practice, owing to the limitation placed on the scope of the method by its cost.

5 With regard to the detail of application of airspray there is still much to learn. In the first place the nature, and distribution in the swathe, of the drop spectrum call for some means of overlapping the large and small drops in order to ensure a more even distribution of the dosage than we were able to achieve. In the method used during these trials it was necessary either to give an unduly large dosage in some parts or to accept an inadequate dosage in others. Wide overlapping of swathes is desirable from the flying aspect, as it increases the ease of application. As an established method, it is obviously desirable that airspraying should not demand too much initiative on the part of the pilot or ground party nor should it be at the mercy of every chance gust of wind. For all these reasons it seems desirable that less than an adequate dosage should be released in each swathe, and that

swathes should widely overlap. On account both of wind variation and of the foregoing considerations a form of spray emission controllable by the pilot is also desirable. If regular airspraying of an area were to be carried out, it could be greatly facilitated by the cutting of permanent base lines obvious from the air, and by the erection of some permanent form of beacon for each run of the aircraft. There is also a need for the simplification of the means of preparation of the aerosol mixture, for solution by heat in the field is a laborious and uncertain method.

6 The foregoing considerations of detail apply in full only to the methods actually used by us. The same qualification applies to the general conclusion that the control effected by the larger dosage of 32 mg per square metre was as efficient as any method of control we have used. It is probably more efficient than the standard practice of larvicidal ground control, and from our experience it is almost certain that aerosol, used in adequate dosage, would control the intense and widespread seasonal breeding of species such as *A. gambiae*.

#### SUMMARY

1 An account is given of the use of DDT aerosol against *Anopheles gambiae* and *funestus* in East Africa.

2 The entomological assessment of the series of sprayings carried out shows that spraying at a dosage of 32 mg per sq metre of pure DDT gave an immediate reduction of *Anopheles* of 98 per cent, but that a dosage of 17 mg per square metre gave results that were little better than standard ground control methods.

3 The effectiveness of these two rates of application followed closely the observed larvicidal action, but there was an unexplained immediate effect on adult anopheline population at the higher dosage.

4 Although this was not conclusively demonstrated, it is concluded that the seasonal peak of *gambiae* breeding was effectively, if not completely checked, this conclusion being based on the slow rate of recovery of vector house catches, and on comparison with previous years.

5 Some substantial evidence is provided that the radius of control against these two species need not in practice be extended, in the fairly typical localities under discussion, beyond 1½ to 2 miles.

6 The main applications of aerosol arise out of the speed and simplicity of its initiation and practice (granted the necessary familiarity with the method), and the capacity of aircraft to overcome ground inaccessibility.

7 Reasons for the comparatively high dosage shown to be necessary were found in the interference to the penetration of the spray to the water surface by vegetational cover, and in the variability of spray distribution attributable to variations in windspeed and direction and to wind turbulence.

8 A further cause for uncertainty in distribution, by the method used, lies in the nature of the drop spectrum of the spray and this can probably be overcome by wide overlapping of swathes at a reduced rate of spray emission.

9 It is considered that airspraying over the limited areas to be covered in practical malaria control is unlikely to cause any serious upset of the biological balance of other fauna.

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## DENGUE IN IRAQ

BY

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The following is an account of an outbreak of a febrile disease encountered in Iraq in the autumn of 1944, in British, Indian and Arab troops in a military hospital. The clinical features and length of illness conform to those of dengue. It appears that there is little or no record of Iraq being an endemic area for dengue fever. On this account, when the cases about to be described began to appear, laboratory investigations were carried out, as far as possible, to determine the exact nature of the illness under observation. These included numerous blood smears in the early part of the illness, total and differential white blood cell counts during and after the illness, blood cultures in glucose broth and bile salt-lactose media, agglutinations against typhoid O, paratyphoid A O, B O, C O, Weil-Felix reactions, OX19, OX2 and OXK, *Brucella abortus* and *melitensis*, Paul-Bunnell, WR and Kahn, routine urine, chest X-ray examinations, intraperitoneal guinea-pig inoculation with citrated whole blood, with examination later of the peritoneal exudate for leptospiral bodies, daily examination of the urine up to 14 days of convalescence, by dark-ground illumination for leptospirae.

Out of a total of more than sixty cases in the present series, careful clinical and pathological observations were completed in twenty-four, who were selected on account of the presence of lymphadenitis. The results of all pathological investigations were negative except for the almost invariable leucopenia, which, with the clinical findings, are in keeping with a diagnosis of dengue.

\* Our thanks are due to Brigadier FOOT for permission to publish this paper, and also to Brigadier T C HUNT for his interest and advice.



There have been in recent years many recordings of 7-day fevers of a closely related character in various parts of the world, all stressing slight differences in symptomatology and clinical signs. Some of these are accepted by their respective authors as dengue proper—others fall into the more general classification of MECAW's "Dengue Sandfly group." Some occurred outside the accepted dengue areas—West Africa (FINDLAY 1943), East Russia (SCHULTZ, 1943), East African Islands (MC CARTHY 1943) North Carolina (DANTELA, 1943). Earlier recordings include the 5-day fever of VAN DER SCHUER in the Dutch East Indies (now accepted by most authorities as a local variant of dengue), red fever of the Congo, Bwamba fever of Uganda, and a 7-day fever described by DREKS in Panama. It appears that dengue is really worldwide in its distribution.

#### CLINICAL FEATURES.

Dengue is an acute infectious disease, caused by a specific virus, and transmitted by the bite of a mosquito. The mosquitoes so far incriminated have been *Aedes aegypti* and *A. albopictus*. The presence of *A. aegypti* in the locality of the recorded cases has been shown in the present series. The mosquito, after sucking infected blood, becomes infective in 11 to 14 days and remains so for the rest of its life. The blood of the patient is infective up to the 3rd or 4th day of the fever and the disease is transmissible from man to man. Dried and frozen serum has been used by HOFFMAN, MARTENS and SVIJDERS to transmit the disease to volunteers injected in Amsterdam, 285 days after the serum had been taken in Java.

The disease is characterized by a sudden onset with chilliness after an incubation period of 5 to 9 days with fever severe frontal or retro-orbital headache, generalized aches and pains, especially in the lumbar and sacral region. The temperature may be continuous with rapid lysis on the 6th or 7th day or may be characterized by a remission about the 4th or 5th day followed by a secondary elevation of 1 to 3 days. There are often associated rashes—a primary 2nd to 5th days and a secondary or terminal usually on the last day of fever often present for only a few hours. The most marked features of the present outbreak were (a) lymphadenopathy and (b) pronounced cough with bronchitis.

#### ONSET

The cases were usually characterized by sudden onset with chilliness—no rigors were recorded. The temperature rose to 104° F and the patient looked and felt very ill. He complained of severe frontal and/or retro-orbital head ache. Many cases complained of occipital headache, which with pain and tenderness down the back of the neck gave a similar picture to the neck rigidity of meningitis. Kernig's sign in such cases was usually doubtful. Lumbar puncture was performed in one such case and in a number of others outside the recorded series. Onset with vomiting and diarrhoea was noted in some. In one this was accompanied by a persistent right lower abdominal pain

without rigidity or tenderness, which remained continuously until the 7th day, when the temperature fell, and the pain disappeared. The diarrhoea and vomiting in this case had gone by the end of the 2nd day.

Pains in the body are a characteristic of dengue, and were a prominent feature of this series—lumbo-sacral or sacral was the most marked and occurred with regularity, and usually with great severity, it was often the first thing of which the patient complained. Pain in one or more joints, or, more usually, generalized aching was combined with backache in many cases.

TABLE I  
SYMPTOMATOLOGY ANALYSIS OF 24 CASES

Symptoms		Signs	
Sudden onset	13	Toxic	0
Chilliness	23	Drowsy	18
Headache	9	Conjunctival congestion	20
occipital	14	Corvza	12
frontal or temporal	18	Tongue furred	20
retro-orbital	16	tremulous	3
Phobias	16	Throat congested	21
Cough	5	Bronchitis (rhonchi)	14
Sputum	7	Flush	16
Vomiting	5	Rash primary	6
Diarrhoea	4	secondary	4
Constipation	18	Spleen palpable	2
Pains in the back (sacral)	16	Tenderness of muscles	22
" lumbar	14	*Glands enlarged	22
" joints	2	posterior cervical	4
" abdomen	17	submaxillary	17
Generalized aches	14	epitrochlear	24
Insomnia	18	axillary	14
Weakness	3	upper mesural	13
Erythema	2	femoral	1
Itching	2	Head retraction	1
Distress	2	Blood pressure (average of 10 cases)	115
		C.S.F. (one case)	2.5

\* In assessing the value of glandular enlargement it must be remembered that only those cases showing enlargement were selected for investigation.

#### SIGNS—PROMINENT FEATURES

*Rash*—The primary rash was macular or maculo-papular and appeared from the 2nd day onward—usually a trace on the 2nd day and more marked on the 3rd and 4th day when it might become widespread. Some of those developing on the 4th day were so profuse that patients were regarded as probable cases of typhus, though the lymphadenopathy tended to distinguish them. The

maculo-papular rash was liable to be confused with a prickly heat eruption on the patient at the same time. The widespread macular rash was easy to recognise as it spread over the chest and back, abdomen, arms, hands and thighs, rarely the legs. One case developed a maculo-papular rash on the 2nd day which became frankly urticarial on the 3rd day but had receded to macular on the 4th. Two similar cases had been seen before this. (The possibility of these cases being schistosomiasis in the invasion stage, the Japanese katayama disease was entertained. However they showed no eosinophilia and gave no history of bathing in infected places and they had the other characteristics of dengue fever.)

The secondary rash appeared on the last day of temperature in all cases in which it was seen, and faded in 2 or 3 days. Occasionally massive scarletiform rashes had been seen in cases outside this series, which showed a few white macules of normal skin on a scarlet background. The usual rash was a macular one distributed over the chest and back, anterior thighs and arms. Over the latter the rash tended to be composed of confluent macules giving an irregular erythematous area. The majority of cases had neither primary nor secondary eruptions. The secondary in some cases was confined to small areas, bilaterally on the anterior thighs and over the triceps area of the arms, near the elbow. If the rash was not looked for in these areas, it was easily missed.

*Bronchitis.*—Practically no mention is made of this in the standard text book descriptions of dengue. It is logical to assume that the intense congestion of the mucous membranes as evidenced by conjunctivitis and severe nasopharyngeal and faucial congestion, with coryzal symptoms, the marked flushing of the face, neck and chest in some cases, would be shared by a congestion of the bronchial mucous membrane. Dry cough, with widespread high-pitched rhonchi heard in the lungs was a feature in many cases, so much so that in the earlier part of the year such cases were dismissed as acute bronchitis, and they then proceeded to run a 7-day fever unresponsive to sulphonamides. The coryzal symptoms were not followed by the purulent nasal catarrh of the common cold.

*Tremulous tongue.*—Tremulous tongue and lips with slight dullness of hearing in an obviously seriously ill patient are always very suggestive of typhus in the 1st week, or typhoid in the 2nd or 3rd week. No other common fever gives this picture so constantly. Several patients showed this, and therefore were regarded as possibly typhus on admission which the appearance of a rash on the 4th day seemed to confirm. Multiple enlarged lymph-glands were of great value in determining the actual diagnosis prior to defervescence or receipt of laboratory reports.

*Glandular enlargement.*—Records of glandular enlargement in dengue vary between "no mention" and 62 per cent. (STITT). Gross epitrochlear enlargement in Europeans is so seldom an indication of disease other than secondary

syphilis and glandular fever that this sign of the disease began to separate them as a group, when it was realized that the enlargement was not associated with a positive Wassermann reaction. Many typical dengue cases have been seen during the period without glandular enlargement but it was decided, as a first step in the identification of the group, to examine carefully only those who showed this feature. There is no doubt that dengue in the summer was more prevalent than was generally realized, if those without glandular enlargement are included.

The commonest groups of glands (not normally enlarged in the healthy individual) were posterior cervical and epitrochlear, and these were therefore a useful guide to diagnosis. Many had two or three glands grossly enlarged in a single site, and not necessarily bilaterally. A gland might be as large as a sparrow's egg, and tender on palpation in the early stages. No glands were aspirated or excised for section, though this might yield information of interest in future cases. They were often not enlarged until the 3rd day, and might therefore be missed unless the case was re-examined. They had usually subsided to normal by the 4th to 7th day of convalescence.

*Splenomegaly*—If the spleen had been palpable during the disease, it disappeared in a few cases. The others remained palpable, and these (all Indian) were thought more likely to have been due to chronic malaria rather than to dengue. Splenomegaly was not a marked feature.

*Bradycardia* was present in almost all cases, very marked in convalescence in some.

*Temperature charts*—The typical saddle-back chart was present in only three of the twenty-four cases, but a composite temperature chart showed that the initial temperature of 102° F (average) was not again reached until the 5th day, when it averaged 103° F, giving a saddle curve to the composite temperature chart. The fever fell to normal on the 6th to 7th days. Cases were met with that seemed to be undoubted dengue, but after the secondary rise of temperature had subsided by crisis, they continued to run a low temperature of 99 to 99.6° F for a further 3 or 4 days. Others that seemed to belong to the same group had fever for as little as 4 days and then developed a terminal rash. There was difficulty in distinguishing such cases from rubella, if the temperature was not greatly elevated. It seems possible that the often stressed 7-day feature of dengue may have to be modified on careful analysis of large series of cases. A more specific means of identifying the disease is needed.

#### LABORATORY INVESTIGATIONS AND X-RAY OF CHEST

##### (TABLE II)

The only feature worthy of note is the more or less constant leucopenia; there was never any absolute lymphocytosis suggestive of true glandular fever and this was confirmed by the uniformly negative Paul-Bunnell tests.

Case number	Nationality	Name	Days febrile	Day of disease blood taken	Total W.B.C.	White blood corpuscles.					Tests.		
						P	L	M	E	B	WR	Hahn	Post-Dumail
1	Arab	R.M.M.	8	5	8,200	71	27	2	0	0	—	—	
				7	4,000	64	28	6	1	1	—	—	8
				22	7,200	66	30	2	1	0			8
2		M.L.	8	5	8,200	71	29	0	0	0			
				8	4,800	62	27	8	2	1	++	+4	8
				23	6,800	62	29	6	4	0	++	+1	4
3	Indon	A.J.	7	8	8,100	64	28	6	1	0	+	—	8
				11	7,800	60	36	10	0	0	++	—	4
4		A.N.	7	8	8,200	82	42	4	1	0			8
				14	8,800	28	62	1	1	0	—	—	8
5		R.	8	4	4,800	28	41	0	1	0			
				16	4,400	58	26	5		1	—	—	16
				18	8,000	80	30	9	1	0	—	—	8
6		S.	6	6	4,080	80	22	8	1	1	—	—	16
				14	6,000	62	28	10	0	0	—	—	8
7		W.M.	12	7	4,200	68	30	4	1	0	—	—	8
				24	8,200	62	30	7	1	0			
8		B.S.	6	8	4,400	64	28	6	2	0	+	—	4
				12	7,080	60	28	10	2	0	—	—	4
9		P.R.	6	4	8,200	62	28	8	1	1	++	+4	8
				12	8,800	68	28	12	2	0	++	+2	4
10		S.	7	12	7,800	86	28	0	6	0			
				21	8,800	62	28	2	1	0	—	—	4
				28									
11		A.D.	7	6	6,000	62	30	7	1	0	—	—	4
12	Brazil	L.	6	6	4,000	62	22	4	1	0	—	—	8
13		W.	8	8	4,800	60	26	2	1	0	—	—	4
				17	6,200	66	28	12	0	0	—	—	4
14		F.	7	2	8,800	62	28	2	1	0	—	—	4
				18	6,000	68	28	10	1	1	—	—	4

phoid O	Paratyphoid			Proteus			Br abortus	Br mel- tensis	Blood culture	Medium	Chest X-ray
	A O	B O	C O	OX19	OX2	OXK					
20 0	0 0	20 0	20 0	0 0	0 0	0 0	0 0	0 0	Sterile	Bile	Normal
20 0	0 0	20 0	20 0	0 0	0 0	0 0	20 0	20 0	"	Glucose Bile	"
0	0	0	0	0	0	0	0	0	—		"
0 20	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	—		—
20 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	—		Normal
20	0	0	0	0	0	0	0	0	Sterile	Bile	"
0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 50	0 25	0 25	"	"
40	0	0	0	0	0	0	0	0	—		—
0	0	0	0	0	0	0	0	0	Sterile	Bile	Normal
20 160 20	0 40 0	0	0	0	0 0 0	0 0 0	0 0 50	0 0 0	0 0 0	—	
40	0	0	0	0	0	0	0	0	Sterile	Glucose Bile	—
20	0	0	0	0	0	0	0	0	—		Normal
20 40	0 40	0 0	0 0	0 0	0 0	0 0	0 50	0 0	0 0	—	"
0 40	0 40	0 0	0 0	0 0	0 0	0 0	0 0	0 0	Sterile	Bile	"

TABLE II-

Case number	Nationality	Name.	Days febrile.	Day of disease, blood taken.	Total W.B.C.	White blood corpuscles.					Tests.		
						P	L.	M.	E.	B.	W.R.	Kahn.	Post-Hemol.
15		B.	7	8 11	4,800 3,800	80 82	24 29	18 8	1 1	0 0	—	—	4
16		M.	6	7 17	8,200 7,200	60 60	28 30	12 10	0 0	0 0	—	—	4 4
17		K.	7	9 12	4,000 3,000	58 58	35 34	6 8	1 0	0 0	—	—	3
18		S.	4	2 11	4,800 7,800	58 62	35 29	5 7	2 2	0 0	—	—	4 4
19		T.	6	2 12	3,800	61	34	5	0	0	—	—	3
20		B.	6	4 13	3,400	62	26	6	1	0	—	—	4
21		A.	7	4	4,000	60	27	7	1	0	—	—	4
22		P.	7	2 15	7,080 6,000	65 60	29 30	6 7	0 2	0 1	—	—	4 3
23	Indian	L.R.	6	4 22	4,800 5,000	48 28	48 62	5 0	1 1	0 0	—	—	4 4
24		S.D.	6	4	3,200	60	23	10	2	0	—	—	4 3

Urine examination in the febrile period showed no evidence of albuminuria.

Dark ground illumination of the urine was carried out on not less than five occasions for each case, up to the 7th day for British, and 14th day of convalescence for the Arabs and Indians. All were negative for leptospirae.

Guinea-pig inoculation (intrapertoneal) with examination of the peritoneal fluid at 7 to 14 days interval was carried out in all twenty four cases with uniformly negative results.

#### DIFFERENTIAL DIAGNOSIS.

Owing to the various manifestations of the disease it has appeared under several diagnoses previously and the following have to be distinguished. The

continued

Typhoid O	Paratyphoid			Proteus			<i>Br</i> <i>abortus</i>	<i>Br</i> <i>mel-</i> <i>tensis</i>	Blood culture	Medium	Chest X-ray
	A O	B O	C O	OX19	OX2	OXK					
0	0	0	0	0	0	0	0	0	—		Normal
0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	—		"
0	0	0	0	0	0	0	0	0	—		"
0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	Sterile	Bile	"
40 20	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	,	,	"
20 20	0 0	0 0	0 0	0 0	0 20	0 0	0 0	0 0	"	"	—
0	0	0	0	0	0	0	0	0	"	,	Normal
20 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	—		—
0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	—		—
0 20	0 0	0 0	0 0	0 0	0 0	0 25	0 0	0 0	—		—

typhus and enteric groups, acute bronchitis, scarlet fever, sandfly fever, influenza, urticaria with fever, rubella, glandular fever, the leptospiral diseases and "clinical malaria," whose rapid "response" to quinine after 2 days' treatment commenced on the 4th day, is all too often regarded as confirmation

*Typhus and typhoid*—The clinical differentiation is discussed above under "tremulous tongue" Typhoid is often enough ushered in with a bronchitis and a leucopenia The leucopenia, a constant feature of dengue, is of value in deciding against typhus One undoubted case with a leucocytosis of 10,000 proved to have a patch of pneumonia on X-ray, and was excluded from this series on that account



## SUMMARY

1 A series of cases of dengue fever occurring in Baghdad, Iraq, are described—they are thought to be the first recorded in this locality

2 The diagnosis from other fevers was made by the finding of a characteristic temperature course, the presence of typical symptoms, and a leucopenia in the blood.

3 Other causes of fever were as far as possible excluded.

4 Adenitis and bronchitis were found to be prominent features.

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## JUNGLE YELLOW FEVER IN SURINAM

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After the publication of the report by SCHUFFNER, WALCH-SORGDRAGER and HOEKSTRA (1938, 1938a) concerning the occurrence of yellow fever in Surinam, the experiments with sera from Surinam were continued at the Institute for Tropical Hygiene at Amsterdam with the intention of testing and adding to the data published in the above-mentioned papers

SCHUFFNER and his collaborators had reached the conclusion, that amongst the inhabitants of the Surinam primeval forest (Bush-Negroes and aboriginal Indians) that form of yellow fever prevails which is independent of the domestic mosquito *Aedes aegypti* and which is known as jungle yellow fever because it is always found in or near the jungle (SOPER, 1936). This jungle yellow fever, as is proved by the results of serological and pathological investigations, usually appears as a disease in adult men, working in the forest or in clearings, whilst the danger of infection is shown to be small for the women and children who

usually remain at home. Only when the house is situated in or very near the jungle is the danger of infection the same for all members of the family. It is therefore accepted that the virus of yellow fever existing in the South American and probably also in the African, jungle is independent of *Aedes aegypti* (which is not found in the jungle) and of human beings who seldom visit it. The most probable hypothesis that other vertebrates and arthropods maintain the virus cycle in the canopy of the primeval forest has been proved correct by the splendid research work of the American investigators during the great war.

#### YELLOW FEVER IN THE SURINAM HINTERLAND

It is shown by the results of the investigations published in 1933, that after the last known epidemic of 1908-09 at Paramaribo (FLU 1910), yellow fever must have prevailed in Surinam. Amongst the Bush-Negroes and aboriginal Indians born after that epidemic, the mouse protection test not infrequently revealed a humoral immunity. It appeared that immunity amongst the male Bush Negroes was more frequent than amongst the Bush-Negroesses (26 per cent. and 4 per cent. respectively). As the virus in these very thinly populated territories seems to exist quite independently of the presence of human beings, we must take it for granted that here we are dealing with jungle yellow fever.

At our Institute immunity is proved by the intraperitoneal mouse protection test according to SAWYER and LLOYD (1931) and by the intracerebral mouse protection test according to THEILER (1931), modified by DIMICK (1931). The last mentioned test is a little less sensitive but requires less serum. With both, the test animals are injected with a mixture of living virus and the serum to be examined. The presence of immune bodies in the serum as a result of an infection by yellow fever virus in the past, is proved by the fact that the mice remain alive. Results of tests may be positive (the donor is immune) negative inconclusive and, finally unsatisfactory in case of early death of test animals (SCHÜFFNER, 1939).

In most cases the total number of sera obtained from one village was far too small to give an insight into the distribution according to sex and age. Therefore we combine the data of all Bush-Negroes and those of all aboriginal Indians (who live quite apart from the Bush-Negroes in the jungle). A discrimination between these two groups may for this reason be justified.

The results of the tests on sera of 128 Bush-Negroes are here recorded, including the data published by SCHÜFFNER *et al.* concerning tests on sera of sixty Bush-Negroes of Ganace on the Surinam river. To these are added the results of tests on three sera of Kraanmanston on the Coppenam river ten of Mabo twelve of Makkakreki twenty-eight of Jacob-kondré ten of Uman-kondré, and one of Granman-kondré all villages on the Saramacca river. Another two sera come from inhabitants of the Surinam river basin.

The results of the serum tests of 126 Bush-Negroes are given in Table I, classified according to sex and age. It shows that amongst seventy-eight men examined, twenty were found immune (26 per cent., probable error 3.3 per cent) and amongst forty-eight women only two ( $4 \pm 1.9$  per cent). Probably the villages of the Bush-Negroes, where the women continually stay, are not contaminated by the virus and the men are infected during their stay in the more distant forest where they carry on their work of cutting trees and managing the freight on the rivers, etc. This concurs with the phenomenon that danger of infection only arises on reaching adult age. It remains remarkable, however, that the Bush-Negress so seldom seems to come in contact with the virus. Although she keeps to her home, the villages are situated in the middle of the forest, and the impression we received from the

TABLE I  
AGE AND SEX DISTRIBUTION OF IMMUNITY TO YELLOW FEVER OF BUSH-NEGROES

Age-group	1-5	6-10	11-15	16-20	21-25	26-30	31-35	51-55	Total
<i>A—Men</i>									
Tested sera	2	9	13	18	24	10	1	1	78
Result inconclusive	0	0	0	2	4	2	1	0	9
Result positive	0	0	2	3	10	4	0	1	20
Percentage positive	(0)	(0)	15	17	42	40	(0)	(100)	$26 \pm 3.3$
<i>B—Women</i>									
Tested sera		4	11	13	6	12	2		48
Result inconclusive		0	0	0	2	0	0		2
Result positive		0	0	1	0	1	0		2
Percentage positive		(0)	0	8	(0)	8	(0)		$4 \pm 1.9$

literature is that under similar circumstances in Brazil the danger of infection is not less for the woman than for the man. Perhaps the virus is localized in areas of the jungle which the Bush-Negress rarely visits or it is carried by a vector which bites during the night, the woman, remaining in the house, might thus be protected. Another remarkable phenomenon is that *Aedes aegypti*—which, according to BONNE and BONNE-WEESTER (1925), prevails in jungle villages such as Gansee—did not spread the virus to the women and children who remained at home. The virus could easily have been imported by a man infected elsewhere.

As to the aboriginal Indians, we have the results of tests of fifty-eight sera, sixteen of which are from Langaman-kondré on the Marowijne river, and already mentioned by SCHÜFFNER *et al*. Of the remaining forty-two, five are from inhabitants of Donderkreek, four from Cornelis-kondré (both on the

Wayombo river), four from Kalebaskreek on the Coppenam, five from the Tibiti savannah (Coppenam) and twenty four from Casipora (Surinam river). The results, classified according to age and sex, are given in Table II. Out of the forty-two men examined, twelve show a positive serum ( $29 \pm 4.7$  per cent.) and four women out of sixteen ( $25 \pm 7.0$  per cent.). It is to be regretted that the material available is rather small, but our data give no indication of a smaller chance of infection for women. SMITH (1939) found in British Guiana, amongst 246 male inhabitants of the jungle 118 immune persons (47.9 per cent.), amongst forty-three female, eight (18.6 per cent.). He does not tell us however how the 174 aboriginal Indians, with sixty-eight immunes (39 per cent.) amongst them, are divided according to sex. In any case our information does not show that the Indian woman is relatively protected against yellow fever virus. This is understandable to some extent if we remember that a family of aboriginal Indians leads a nomadic life. Although it is difficult to

TABLE II.

AGE AND SEX DISTRIBUTION OF IMMUNITY TO YELLOW FEVER OF ABORIGINAL INDIANS.

Age-group	6-15	16-25	26-35	36-45	46-55	Total	Men. Total.	Women. Total.
Men and Women. Tested sera ....	16	20	9	10	3	58	42	16
Result inconclusive	2	1	1	0	0	4	3	1
Result positive	3	6	3	3	3	18	12	6
Percentage positive	18	30	(22)	30	(67)	38	$29 \pm 4.7$	$25 \pm 7.0$

prove it is very probable that the kind of yellow fever which prevails amongst Indians is jungle yellow fever. We would certainly expect higher immunity values with infection by *Aedes aegypti*.

It is worth while considering whether the epidemics that have prevailed in the capital, Paramaribo in 1902 and 1908-09 have also penetrated the jungle. SCHÜFFNER *et al.* already thought this could be denied and our data confirm this. Of twenty-six Bush-Negroes and aboriginal Indians, living in the forest during the epidemics in the capital, nine were immune ( $35 \pm 9.2$  per cent.). Of sixty-one former inhabitants of Surinam, who were living at Paramaribo during one of the two epidemics and are now staying in the Netherlands, forty ( $66 \pm 6.1$  per cent.) were immune. Immunity proved here to be nearly equally divided between men (twenty-six of thirty nine or 67 per cent.) and women (fourteen of twenty two or 64 per cent.). Immunity rates differ considerably ( $31 \pm 11.1$  per cent.) when we compare inhabitants of the town with those of the jungle. This fact renders it improbable that

penetration of the primeval forests by the known epidemics has occurred, the more so because the percentage of 35 concurs with the data of the jungle infection as shown in Tables I and II

#### YELLOW FEVER IN THE CAPITAL AND IN THE "DISTRICTS"

So much for the inhabitants of the forest, who evidently continually run the risk of being infected. Is this also the case with the coastal population, the inhabitants of Paramaribo and those who live in the "Districts," the agricultural region near the coast? It is mentioned in the report of 1938, that twenty-three out of 178 sera (13 per cent), received from Surinam between 1935 and 1937, were positive. These sera came from inhabitants of Paramaribo and "districts," who were not living there during the known epidemics. It seems difficult to accept the supposition that danger of infection with the yellow fever virus existed after 1909 in the capital of Surinam or in its close vicinity. If this had been the case, the presence of many sensitive persons and the numerous *Aedes aegypti* ought to have caused an epidemic. Such has not been observed since 1909 and it seems most unlikely that an epidemic could have occurred without being recognized. It is much more probable that the danger of infection exists in the "districts," but then the question arises of the more exact localization of the virus.

The data of 1938 made it necessary in the future to distinguish between persons who were practically always living in the capital and those who lived in the "districts." We are now able to report on the tests of the sera belonging to the first-named category.

In 1939, through the good offices of Dr TILLEMA and Dr WOLFF (who also procured many other sera from Surinam), our Institute received the sera of a number of school-children from Paramaribo. Although the schools are situated in the town, most of the children have their homes in the neighbourhood. Most of them are children of British Indians, small farmers in the neighbourhood. Of ninety tested sera, none were found to have protecting properties against yellow fever virus. We also have data of thirty-six former inhabitants of Surinam, now living in the Netherlands, who were not at Paramaribo during the known epidemics. They all lived in the capital for the greater part of their stay, several of them visited the hinterland and some served at a certain time of their life on sea-going ships of the Merchant Navy. It would not have surprised us, therefore, if one of the sera should have been found to be positive. As this was not the case, we believe that we may use the results of the tests of all these thirty-six sera for judging the danger of infection, which might have existed at Paramaribo since 1909.

After comparing the results obtained before and after 1938 (Table III), we think we may conclude that there is no reason to suppose that epidemics of yellow fever have occurred at or very near Paramaribo after 1909. It is, however, very probable that the "districts" have been infected. We have no reason to

suppose that the agricultural population along the coast ran the risk of infection only by visiting the interior. Thus in our opinion, would not happen with sufficient frequency to explain an immunity rate of more than 10 per cent. We therefore conclude that yellow fever remained smouldering in the coastal regions also after 1909. The question remains whether jungle yellow fever is here playing its part or whether the virus has exclusively maintained itself thanks to the cycle man-*egypti* man. On the plantations along the coast *Aedes aegypti* is very often observed. This mosquito will certainly if the virus is present, be able to cause epidemics, which have, however never been observed, though the possibility of their having occurred must still be considered. It does not, however seem probable that man and *aegypti* together could form the reservoir of the virus in the "districts." Infection there is probably endemic only very special circumstances in Brazil created the possibility of endemic existence of rural yellow fever carried by *Aedes aegypti* (SOPER, 1938).

TABLE III

IMMUNITY TO YELLOW FEVER OF INHABITANTS OF THE COASTAL REGION, NOT LIVING IN THE CAPITAL DURING THE EPIDEMIC.

	Inhabitants of Paramaribo and districts (research 1935-37).	Inhabitants of Paramaribo and close vicinity (research after 1938).
Number of sera examined	178	128
positive sera	23	0
Percentage positive sera	13	0

It is also possible that inhabitants of the districts are occasionally infected by yellow fever virus which as a rule has other hosts and, in accordance with experience gained elsewhere, probably comes from the forest. A great part of the coastal region is also covered by forest, and many plantations immediately adjoin it. The occurrence of jungle yellow fever in the coastal region is thus quite possible. We cannot, however prove this as data are wanting as regards the places in which those we regard as immune have been infected. It is improbable that the whole coastal region could be one large focus of jungle yellow fever. It can more easily be accepted that the danger of the occurrence of this disease is local. The surest way of localizing these supposed foci of infection would be by the introduction of systematic viscerotomy in Surinam, as has already been done in South American countries. Viscerotomy is employed as a routine postmortem measure for the removal of liver tissue for histological examination from all persons who die within 10 days of the onset of their fatal illness. The object of organized viscerotomy is to obtain information regarding

the presence or absence of yellow fever in a definite region In Brazil the application of systematic compulsory viscerotomy has considerably promoted the yellow fever research (SOPER, RICKARD and CRAWFORD, 1934, RICKARD, 1937)

The problem we met among the inhabitants of primeval forests was, that there was no sign of the jungle yellow fever virus penetrating the Bush-Negro and Indian villages Neither do we see any signs of infection of the coastal regions by *Aedes aegypti*, although this mosquito is abundant on the plantations and in the town and villages - In other words no epidemics of yellow fever occur on the coast although sensitive persons and the vector are present and the virus is not far away Although jungle virus can penetrate a town (WALCOTT, CRUZ, PAOLIELLO and SERAFIM, 1937), this happens less often than one would expect, and this has also been the experience elsewhere We cannot yet give a definite explanation of this phenomenon

### CONCLUSIONS

Serological research continued after 1938 shows that we must distinguish three regions in dealing with the yellow fever problem in Surinam (POLAK, 1944)

A The hinterland covered with primeval forest, inhabited by Bush-Negroes and Indians Here yellow fever prevails in its jungle form Human infection is relatively frequent, though Bush-Negresses rarely seem to come in contact with the virus

B The agricultural regions on the coast with plantations situated near the forest Here also human infection seems fairly common The yellow fever we meet is probably mostly jungle yellow fever

C The capital, Paramaribo, is in close contact with the agricultural region and is yearly visited by many inhabitants of the jungle There are no indications that yellow fever has prevailed here after 1909, but the penetration of the virus is certainly possible and the appearance of a severe epidemic would then be expected Control of *Aedes aegypti* and preparation of mass vaccination are very desirable

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# STUDIES IN LEISHMANIASIS IN THE ANGLO-EGYPTIAN SUDAN

## IX FURTHER OBSERVATIONS ON THE SANDFLIES (*PHLEBOTOMUS*) OF THE SUDAN

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### INTRODUCTION

A study of the sandflies (*Phlebotomus*) of the Sudan was started by the writers in 1938, but was interrupted by the war. For the past 6 years our attention has been fully occupied with other matters, and we have been unable to pursue a systematic study of *Phlebotomus*. During this period, however, collections have been made from time to time as opportunity arose, and examination of the material thus obtained has considerably increased the records of species of *Phlebotomus* in the Sudan. Such records naturally include odd observations on methods, distribution, bionomics, and similar matters. The present communication is an attempt to collect the rather disjointed

\* We are grateful to those who have sent to us collections of sandflies from different parts of the country, particularly Mr J W COWLAND, Dr A R HUNT, Dr R W STEPHENSON and Dr C M MACDONALD, to Dr L PARROT of the Institut Pasteur Algérie for the determination of *P. alexandri* from the Sudan, also to the DIRECTOR SUDAN MEDICAL SERVICE for permission to publish this paper.



comparing them with type specimens become available to us. Records of distribution and other observations noted in our earlier paper are not included in the present article, in which only additional localities are given.

### METHODS

Preliminary studies, recorded in our previous paper, directed attention to the bionomics of *Phlebotomus* in outdoor situations rather than in houses and towns. Many of our observations have been made in the wilderness, in native villages, temporary camping grounds, cattle camps and similar places, which are often only seasonal oases in the wilderness. The methods used in collecting have been similar to those recorded in our earlier paper, with some additions.

The use of oiled paper traps, previously described, has been found an invaluable method, especially for obtaining collections from holes and cracks in the ground, burrows of animals and birds, termite mounds, clefts in trees, caves, rock crevices and similar places. The viscosity of the oily film is just sufficient to hold sandflies and other equally small creatures with feeble powers of flight. Most beetles, mosquitoes, bugs and larger insects generally break away, and so do not feature in the catches. The main point in the use of these traps is knowing where to put them, and with increasing knowledge of the habits of sandflies one learns to choose the likely places. Occasionally some species are so numerous that traps placed almost anywhere will catch them. To collect sandflies in rooms, caves, huts, and in the open before the war we devised traps made of cones or cylinders of oiled paper illuminated inside by small electric torches, but the torches have been unobtainable during the war, and we have ceased using these traps, and found simple oiled paper traps satisfactory. We found it was often possible to catch sandflies by simple traps in cleft sticks placed in suitable places in houses, etc.

Baited traps were also found extremely useful, especially for catching living sandflies. These traps consisted of tents made of sandfly netting, with a caged animal inside, and placed generally on the ground. With scissors, a series of small holes was cut round the tent, about half-way between ground level and the top of the tent. We found that sandflies readily entered such a trap through the holes, but seemed to have difficulty in finding their way out again, since reasonable catches could usually be collected in the early morning. The sandflies which had fed were usually found gorged in the corners at the bottom of the net, while those which had not fed seemed to miss the exit holes and get lost in the corners at the top. It is, of course, important to ensure that the trap is closed below (between the animal and the ground) by a layer of cloth impervious to sandflies, and that the junctions between this and the tent are also impervious to sandflies.

A further development of this method used by us was to cut holes about half-way up the sandfly net of a human volunteer and, in the same way, collect the sandflies from his net in the morning. When it is desirable to obtain the

sandflies in a viable condition we found it necessary to collect them in the early morning, before they had been subjected to the effects of the sun, which appears to be inimical to them.

#### SANDFLIES HITHERTO IDENTIFIED.

In 1940 we published a list of thirteen species and varieties of *Phlebotomus* which were known in the Sudan at that time. Twelve more have now been found and are indicated by asterisks in the following list of the twenty five named forms.

<i>P. langeroni</i> var. <i>orientalis</i> Parrot.	<i>P. signatipennis</i> Newstead.
<i>P. longipes</i> Parrot and Mart n.	<i>P. similis</i> Newstead.
<i>P. sergenti</i> var. <i>sacrus</i> Parrot and Martin	<i>P. schouteden</i> Adler Theodor and Parrot.
<i>P. alexandri</i> Sinton.	<i>P. congolensis</i> Bequaert and Walravens.
<i>P. martini</i> Parrot.	<i>P. congolensis</i> var. <i>distinctus</i> Theodor
<i>P. papatasi</i> Scopoli	<i>P. africanus</i> Newstead.
<i>P. papatasi</i> var. <i>bergeroti</i> Parrot.	<i>P. africanus</i> var. <i>sudanicus</i> Theodor
<i>P. ouboudi</i> Newstead.	<i>P. africanus</i> var. <i>niger</i> Parrot and Schwetz.
<i>P. rodhaini</i> Parrot	<i>P. schwezei</i> Adler Theodor and Parrot.
<i>P. adleri</i> Theodor	<i>P. schwezei</i> var. <i>aethiopicus</i> Parrot.
<i>P. affinis</i> Theodor	<i>P. decipiens</i> Theodor
<i>P. clydei</i> Sinton.	<i>P. ingrafi</i> Newstead.
<i>P. aquanilepis</i> Newstead.	

#### NOTES ON THE SPECIES

##### 1. *P. langeroni* var. *orientalis*

From a review of the data then available, ADLER and THEODOR (1931) concluded that the distribution of kala-azar in the old world is related to that of sandflies of the *major* group, and predicted that a sandfly of this group would be found in the Sudan, the distribution of which agreed with that of kala-azar and in which the parasites undergo specific development. *P. langeroni* var. *orientalis* is the only sandfly of the *major* group whose distribution is related to that of kala-azar in the Sudan, and preliminary (unpublished) observations have revealed anterior development of the parasites of Sudan kala-azar in small proportion of gorged specimens of *P. langeroni* var. *orientalis* which had fed on patient with post-kala-azar dermal leishmaniasis.

In most places where close correlation between the distribution of kala-azar and that of sandfly of the *major* group has been demonstrated, the sandfly concerned is one of the commonest species in the region and can be readily caught in large numbers. This is not so in the Sudan with *P. langeroni* var. *orientalis*, which is erratic and variable in its occurrence. When found, it is usually outnumbered by other species in the same area, in collections made by the methods described. This is not entirely to be explained by restricted seasonal incidence. In 1942 we found *P. langeroni* var. *orientalis* in considerable numbers in a place where we had found only an occasional specimen in 1941 at the same time of the year and under the same conditions. Possibly these facts may have some bearing on the erratic and variable occurrence of cases of kala-azar in the Sudan. It has sometimes been noticed that a place which produced cases in one year appeared in

the following year to be free from the infection which had instead broken out in some other place

Distribution El Hamra

### *P. longipes*

*P. longipes* is like *P. langeroni* var *orientalis*, a member of the *major* group, but its distribution in the Sudan is not related to that of kala-azar, as far as present records indicate. The species has been found only in one locality in the Sudan, at an altitude of 6,500 feet, in the rain forests on the Imatong Mountains. This locality is practically uninhabited, there are no records of leishmaniasis having occurred there, and the climate is entirely different from that of the kala-azar areas on the plains. The only other record of this species is from Addis Ababa where the altitude is also high.

The members of the *major* group having a non-bifid intromittent organ (*P. langeroni* and its variety *orientalis*, *P. longipes* and *P. longicuspis* Nitz) are separated from each other only by very minor taxonomic differences, while the last species is so closely related to *P. perniciosus* that PARROT (1936) had to resort to breeding experiments in order to satisfy himself that the two were distinct. All these species are, therefore, very closely related to the vectors of Mediterranean kala-azar (ADLER, THEODOR and WITENBERG, 1938), and PARROT (1941) has shown that in experimental conditions *P. longicuspis* becomes as easily infected with canine leishmaniasis in North Africa as does *P. perniciosus*.

Distribution Gilo (6,500 feet, v 45, 6 ♂♂, 1 ♀)

### *P. sergenti* var *saevus*

Distribution Katire (v 45, 1 ♂)

### *P. alexandri*

Distribution Lotelope (v 45, 1 ♂) Loelli (v 45, 2 ♂♂)

### *P. martini*

This species presents features of both *major* and *sergenti* groups. The shape of the distal segment of the superior clasper of the male, and the number and arrangement of the spines on this segment resemble the *major* group. PARROT (1936), however, refers *P. martini* to the *sergenti* group, on account of the pedunculated tuft of hairs on the proximal segment of the superior clasper. Similar features are found in two other Ethiopian species which are known only by the males, *P. katangensis* and *P. rossi*. They are obviously closely allied to *P. martini*. Both have been described as members of the *major* group, *P. katangensis* by THEODOR (1933) and *P. rossi* by DE MEILLON and LAVOPIERRE (1944), and suggested as possible vectors of leishmaniasis and other conditions, although the places in which they were found lie outside the recorded distribution of leishmaniasis in Africa. In the Sudan *P. martini* has been found only in the Southern Sudan, in places round about the southern endemic area centred at Kapoeta. Large numbers of this species have not been taken so far. Most of the specimens have been obtained by oiled paper traps from the burrows of animals, principally porcupines, and the species has not yet been caught in the act of biting man, although it may well do so in suitable conditions.

Distribution Kapoeta, Lotelope (v 45)

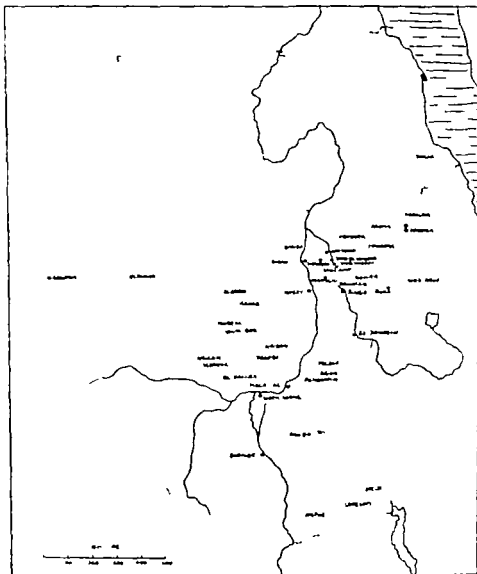
### *P. papatasi*

This species is often found in houses, but on one occasion was found biting in a dried-up swamp 800 metres from the town of Rahad. It has occasionally been found biting on roofs about 6 metres above the ground.

Distribution Abu Guta (J W COWLAND), Abu Usher, Alapatun, Aroma, Bagiga (COWLAND), Damali (A A BEREIR), Damasim, El Hamra, Kassala, Kost, Lul, Omdurman, Rahad, Shambat

### *P. papatasi* var *bergeroti*

Distribution Aroma (iii 43), Kassala (ii, iii, 43)



MAP II.—ANGLO-EGYPTIAN SUDAN.

Showing places mentioned in this paper from which *P. cyanea* has been obtained.

*P. roubaudi*

This species was described by NEWSTEAD (1913), but may be the same as *P. dubosqi*, a species previously created, but incompletely described by NEVEU-LEMAIRE (1906), PARROT (1943) considers this species to be the probable vector of oriental sore in the Niger basin. No cases of cutaneous leishmaniasis have been reported from the only area in the Sudan in which we have found this species.

Distribution Kapoeta, Lotelope (iv, v 45)

*P. rodhami*

The morphology of the male genitalia is highly characteristic in this species which was described by PARROT (1930) from the Belgian Congo (male only). Our specimens differ from PARROT's description in the shortness of the fourth palpal segment, the formula being 1, 4, 2, 3, 5, and the average relative lengths of the segments in five males being 1—2.4—3.6—2.0—4.6. One female of this species obtained by us had a similar palpal formula, the relative lengths being 1—3.0—4.3—2.1—5.0.

Distribution L1 Rangu (xii 41), Kapoeta (iv 45), Sources Yubu (v 40, A. R. HUNT)

*P. adleri*

Distribution Abri, El Fasher, El Obeid, Kassala, Keilak, Kortala, Port Sudan

*P. affinis*

The species *P. affinis* was described by THEODOR (1933) from one female caught by Dr ROSIE in Mongalla province. It has not subsequently been found there, but we have found the species on several occasions in collections from Fasher. The male was described by us in 1940. A feature commonly observed in the females from Fasher and not mentioned by THEODOR in his original description is the presence of numerous spines projecting inwards from the lateral walls of the buccal cavity anterior to the buccal armature.

*P. clydei*

*P. clydei*, which falls into the subgenus *Sintonius* (cf KIRK and LEWIS, 1946), was originally described by SINTON (1928) from specimens from Waziristan and has since been found to be widely distributed in the plains of India (SINTON, 1932).

In 1939 LEWIS and KIRK recorded this species from Africa and noted that it was widely distributed in the Sudan. In 1944 PARROT and MARTIN recorded the species from Djibouti, and stated that they consider *P. ragus*, previously described by them (1939) from Abyssinia, to be a synonym of *P. clydei*. In 1945, PARROT, MORNET and CADENAT recorded *P. clydei* from Dakar and other places in French West Africa, commenting on the wide distribution of this species in Africa extending from the Somali coast (Djibouti) in the east to the Atlantic in the west.

The methods of collection used by us have shown *P. clydei* to be one of the commonest and most widely distributed sandflies in the Sudan, its distribution, which is shown in Map II, page 874, corresponding roughly with that of leishmaniasis. In view of this, it is surprising that the species is not recorded in the collections made by KING, ARCHIBALD, HENDERSON and others who had previously made quite extensive collections of *Phlebotomus* in this country. We believe that the absence of *P. clydei* from the records of earlier workers in the Sudan is due to the fact that *P. clydei* is essentially an outdoor species. It rarely comes inside houses and similar places although large numbers can often be caught in the wilderness by the methods we have described. Probably in the wilderness it feeds largely on burrowing animals, as it is the sandfly which we have caught most constantly from animal burrows.

In preliminary (unpublished) experiments the development of flagellates has been observed in one out of forty specimens of *P. clydei* which had fed on a patient with post-kala-azar dermal leishmaniasis but the development was not of the "anterior" type.

Distribution Abri, Abu Guta (COWLAND), Abu Usher, Agor Ti, Akobo (R. W. STEPHENSON), Aroma Bagiga (COWLAND), Damasin Doka, El Amir, El Hamra, Heiban, Jebel Mandera (F. B. BELL), Kapoeta, Kassala, Katcha (C. M. MACDONALD), Keilak, Kortala, Kosti, Ler, Loelli, Lotelope, Malakal, Merebea (E. JANE), Paloic, Panamtin, Rahad, Shambu, Talodi, Tokar (x 12 H. H. KING), Umm Ber, Wath Wang Kech



*P. squamipennis.*

This species was described originally by NEWFIELD (1912) from a single female from Khartoum. Later STROTT (1925) recorded the species from India and described the male and female, adding in 1928 the description of the characteristic buccal armature. *P. squamipennis* has since been found to have a wide distribution in Africa and Asia, and varieties have been separated on characters in the female, var. *indicus*, var. *dryoides* and var. *sumatrensis*. We have observed in some of our specimens from the Southern and Eastern Sudan a feature which is not described in any of these varieties, nor in the type, viz., the presence of a row of anterior punctiform teeth in the buccal armature of the female. The species has only been found in small numbers except in collections made near lights in houses and in holes in the ground in forests (Table I, page 880). It should be noted, however, that at least four other species were found in one of the forests, on objects in the vicinity.

Distribution Abu Usher and Akobo (STREPTONOS) Alapetun, Bagira (COWLAND) Boin (STREPTONOS) El Amira, Fanjak, Juba, Keilak, Korti, Lul, Loeli, Lotelopi, Malakal, Merebes (JACO) Qala en Nahl, Rashad, Wad el Magdub, W. th Wang Kech, Yel.

*P. signatipennis.*

In our experience, this is the commonest and most widely distributed species in the Sudan, being everywhere easily obtainable in latrines, empty houses, tree clefts, holes in the ground and similar places. It was recorded in our earlier paper as *P. sumatrensis* var. *signatipennis* but from PARROT's (1942) more recent paper the correct designation appears to be *P. signatipennis*.

We have observed that individuals of this species often vary considerably in size also in the shape of the buccal cavity and, more especially the pharynx of the female, but we are unable to state the extent to which the latter differences are due to artifacts arising in the processes of clearing, mounting and viewing. It may be noted that some of the aberrant appearances resemble the descriptions of *P. sumatrensis* (GALLIEN and NITTELEND 1931), *P. schottlandi* var. *antennatus* (PARROT 1930), which are now regarded by PARROT (1942) as synonyms of *P. signatipennis*, and also *P. dentata* (SPROTT, 1933) which appears to be a closely allied species.

Distribution *P. signatipennis* has been obtained from the following: Abu Usher, Aroma, Bagira (COWLAND), El Amira, Erkowit (COWLAND), Hadaliya, Heben, Kaduqli (H. W. BEDFORD), Katcha (MACDONALD), Keilak, Kortala, Korti, Juba (A. M. BROWN), Merebes (JACO) Nuri (COWLAND), Qala en Nahl, Rahad, Tubur Unm Ber. The variation of the pharynx noted above has been seen principally in specimens taken from the Southern Sudan at the following places: Agor Ti, Akobo (STREPTONOS) Alapetun, Boin, Juba, Kaporta (A. E. LORENZEN), Katcha (MACDONALD), Keilak, Kortala, Ler, Loeli, Lotelopi, Malakal, Paloch, Panamta, R. Zeraf (121 km. from mouth) Tiptap W. th Wang Kech.

*P. sumatrensis.*

Distribution Katre (45) Sources Yubu (many) viz. xi. 40, HUNT.

*P. schottlandi.*

Distribution Amadi (xi. 41), Katre (45), Sources Yubu (vi. ix. 40 HUNT).

*P. congoensis.*

Distribution Kaporta, Yel.

*P. congoensis* var. *distinctus*

This variety is widely distributed but is usually found in small numbers.

Distribution Abri, Amadi, Damsat, Dinder, El Obeld, Gallabat, Heben, Juba, Katcha (MACDONALD) Li Rangu, Meridi, Rashad, Yambio, Yel, Wad el Magdub.

*P. africanus*

*P. minutus* var *africanus* was originally separated from the type by NEWSTEAD (1912) on features of external morphology. Later, ADLER and THEODOR (1926), in their classic paper, raised the variety to specific rank as *P. africanus* Newstead, and since that date many varieties of *P. africanus* have been described. *P. africanus* and its varieties are, in our experience, almost as abundant and ubiquitous in the Sudan as *P. signatipennis*, specimens being found in all kinds of habitats.

Distribution *P. africanus* and its varieties are widely distributed in Africa, and occur also in the Mediterranean region and also in parts of Asia. In the Sudan the distribution of *P. africanus* is much the same as that of *P. africanus* var *sudanicus*, which is given in detail below.

*P. africanus* var *sudanicus*

In our previous paper we described a curious habit of *P. africanus* of congregating in large numbers on tree trunks at dawn and sunset, which was observed in uninhabited country at Wad Arud, on the upper reaches of the river Atbara. This habit has been noted in *P. africanus* var *sudanicus* at Hawata, Buffalo Cape (Table I) and other places, most of the insects being females.

Distribution Abri, Abu Tong, Agor Ti, Alapatun, Adok, Amadi, Buffalo Cape, Dmer, El Fasher, Fanjak, Gedaref (COWLAND), Heiban, Juba (BIRREL), Kassala, Keilak, Lake No, Ler, Malakal, Paloic, Panamtin, Qala en Nahl, Rahad, R Zeraf (190 km from mouth), Sennar, Sources Yubu (HUNT), Talodi, Tendelai (COWLAND), Thar Jath, Tiptiap, Wath Wong Kech.

*P. africanus* var *niger*

Distribution Amadi, Li Rangu, Meridi, Sources Yubu (HUNT), Ye.

*P. schwezei*

Although widely distributed, this species has only been found in large numbers in collections from a tree hole (mostly females) and an aviary (mostly males). The significance of these findings is unknown.

Distribution Abri, Abu Usher, Atbara, Dingba, El Hamra, El Obeid, Fanjak, Hawata, Kassala, Katire, Keilak, Kortala, Lake No, Li Rangu, Paloic, Qala en Nahl, Zeraf (190 km from mouth), Roseires, Sources Yubu, Tubor, Um Ber, Yambio, Ye, Wath Wang Kech.

*P. schwezei* var *aethiopicus*

Distribution Fanjak, Hawata, Sources Yubu (HUNT), Wad el Madgub, Wad Medani.

*P. decipiens*

Distribution Li Rangu, Sources Yubu (HUNT), Abu Tiga, Yambio.

*P. ingrami*

Distribution Kagulu, Katire, Sources Yubu (HUNT), Yambio, Ye.

## VARIATION IN COLOUR

When we first examined sandflies from the Southern Sudan we immediately noticed that some of the species were much darker than those further north, and that in several species southern specimens had such dark chitin that the pigmented area of the buccal cavity was opaque and rendered the buccal teeth almost impossible to see unless they were dissected out. These specimens were so dark that it appeared that they might be regarded as varieties. It

seems probable, however that they merely illustrate the tendency in many groups of animals for desert forms to be unusually pale, a tendency which should be readily noticeable in the Sudan with its gradation from wooded country to pure desert. CORR (1940) writes "The correlation between dark pigment and high humidity and between pale colouration and arid conditions, has been long recognized, not only among different mammals and birds but also in various reptiles and insects." He and also HUXLEY (1942) discuss the causes of desert pallor. Dark southern sandflies include *P. similis*, *P. decipiens*, *P. africanus* var. *niger* and a species not yet described, and intra-specific dark variations are seen in *P. schouteni*, *P. congolensis* and its variety *distinctus*. LEWIS (1945) records that in several species of mosquito in the Sudan northern specimens show pale variations. These observations on Sudan sandflies and mosquitoes may be comparable to NASH's (1937) observations on intra-specific and intra-generic colour variations in tsetse flies in Nigeria.

#### FEEDING HABITS.

The following sandflies have been taken in the act of sucking blood, chiefly in houses in various parts of Northern Sudan —

	Man	Gecko
<i>P. papatasi</i>	23	—
<i>P. clydei</i>	5	—
<i>P. signatipennis</i>	—	9

Hundreds of *P. papatasi* could have been collected, but the main intention was to collect sandflies which appeared to belong to other species. *P. clydei* has been found biting by day and night, but the number of records of this species biting in houses is small. The number of ungorged females in the net at Hawata (Table I col 27 p. 880) showed that this species bit man less readily than did *P. langeroni* var. *orientalis* on that occasion. Near Kosti, in October one of us (D. J. L.), when collecting mosquitoes on cracked cotton soil 600 yards from the Nile after dark was attacked in a few minutes by scores of sandflies which proceeded up the body by jumps and bit chiefly on the arms and neck. Of eleven caught and identified, all were *P. clydei*. Mammalian blood has been found in specimens of *P. clydei* taken in oiled paper traps from the burrows of foxes and ground squirrels in Singa and at Hawata we obtained three gorged females of this species in a net trap baited with ground squirrels. In a net trap baited with a monkey at Gedaref a female of *P. papatasi* and one of *P. schouteni* were found gorged with blood.

Our present information on the biting habits of sandflies in the Sudan may be summarized as follows. *P. papatasi* bites man very readily indoors, and has been found biting out of doors. It will probably bite a monkey in a net trap. *P. papatasi* var. *bergeroti* has not been seen biting man, but MARTIN (1938) thinks it probably bites in Abyssinia. We have no records of *P. roubaudi*.

biting man. Neither *P. martini* nor *P. longipes* have been caught in the act of biting man by us, but we have obtained only a few specimens so far. Both are recorded by MARTIN (1938, 1939) as biting man in Abyssinia, who cites also dogs, rabbits, guinea-pigs, fowls and pigeons for the latter species. *P. lanigeroni* var. *orientalis* bites man very readily. *P. clydei* bites man in and out of doors, but is principally an outdoor species. *P. squamipleuris* has not been seen biting; WANSON (1942) has identified lizard's blood in its gut. *P. signatipennis* commonly bites gecko lizards, but seldom or never bites man. We have observed a fairly constant association between *P. signatipennis* and lizards. This species has been caught in the act of biting lizards, and lizards have been observed catching and eating sandflies where collections revealed only *P. signatipennis*. Twenty-three small sandflies were observed on a single gecko on the outside of a house at Wad Medani at 10 p.m. in June. Although SINTON (1932) records *P. congolensis* as biting man in Kenya, the absence of similar records from the Sudan is not surprising since this species has not been found in large numbers, var. *distinctus*, though widespread, is seldom abundant. It would be interesting if the Kenya observations could be repeated, since the collection of *P. congolensis* received by SINTON and reported as biting appears to have included males. *P. africanus* bites man rarely; WANSON (1942) showed that it bit man and lizards. In the Sudan we have not caught *P. schweizeri* in the act of biting man, although it apparently does so in the Belgian Congo (THEODOR, 1931), and we have received specimens taken on man in Mombasa from Mr C. TEESDALE of the Health Department. This species will probably bite monkeys in a trap. WANSON (1942) has seen it biting man and showed that it would feed on fowls, rabbits, bats and lizards. MARTIN (1938) thought that *P. schweizeri* var. *aethiopicus* probably fed on man. A male *P. papatasi* has been observed apparently trying to insert its proboscis into the leaf of a sedge plant standing in a vase.

#### PARASITES

In addition to the records given in our previous paper mites have been found on specimens of *P. clydei*, *P. schweizeri*, *P. martini* and *P. congolensis* var. *distinctus*. Fungus infection has been found in specimens of *P. ingrami* and a species not yet described. In both cases the principal site of the infection appeared to be the female genital tract, ovary, spermatheca and their ducts. In a male provisionally determined as *P. adleri* the body cavity was found densely packed with bodies shaped like pseudonavicellae having a well defined wall and a central globule of highly refractile material. It was at first thought that these were probably fungal spores, although no medium could be seen. But their reaction to the clearing and mounting media used for this specimen was different from that of fungi, so their nature is unknown. Nematodes have not so far been observed in any specimen. Occasionally we have been stranded in out-of-the-way rest houses in which in the

TABLE I  
SHOWING NUMBERS OF SANDFLIES IN SOME COLLECTIONS FROM VARIOUS SITUATIONS. FEW OLD COLLECTIONS ARE INCLUDED EXCEPT WHERE  
THEY WERE SET. THE TRAPS ARE ALL TRAPS USED A NIGHT. SEES ARE INDICATED ONLY WHERE THE NATION APPEARED TO ME OF PARTICULAR INTEREST.

Species	Karido- grad Forest, 7.11.39 ground holes	Wad el Mashub Forest, 21.8.39 ground holes	Abou Usher, 27.5.42, near light in house	Suqat, 22.10.39 near light	Wad Madani, 1938 near blue light of refrigerator	Thar Jeth, 4.1.40 trees in morning	Hawata, 17.11.39 on tree by day	Buffalo Cape, 3.1.40 on tree in morning	Palosa, 15- 18.10.39 on tree by day
<i>P. papillata</i>	—	—	0	—	♂ 1	—	—	—	♂ 1
<i>P. longirostris var. orientalis</i>	—	—	—	—	—	—	—	—	—
<i>P. (P.) di-</i>	—	—	1	—	—	—	—	—	—
<i>P. apicalifrons</i>	23	13*	104	23	64	—	—	—	—
<i>P. equidens</i>	—	—	12	1	7	—	—	—	—
<i>P. apicalifrons</i>	—	—	—	—	20	—	—	—	—
<i>P. equidens</i>	—	—	—	—	—	—	—	—	—
<i>P. africana</i>	—	—	—	—	—	—	—	—	—
<i>P. africana</i>	—	—	—	—	—	—	—	—	—
<i>P. africana</i>	—	—	—	—	—	—	—	—	—
<i>P. typ.</i>	—	—	—	—	—	—	—	—	—
Total	23	13	123	24	71	29	17	40	8

8 other species on near-by trees and between hall.

TABLE I—continued.

Species	Lar on wooden post by day	Wad Medani, 21.11.39 avary	Wad el Mashub Forest, 1.8.39 hole in tree	Palosa, 17.11.39 ground hole by day	Senar 2.8.39 in house by day	Tripoli 25.12.39 ground traps	Wadi Kech, 1.1.40, ground traps	Palosa, 22.12.39 ground traps	Kerik, 14.12.40 traps hole and tree
<i>P. papillata</i>	—	♂ 1	♂ 1	♂ 1	—	—	—	—	—
<i>P. longirostris var. orientalis</i>	—	—	—	—	—	—	—	—	—
<i>P. (P.) di-</i>	—	—	—	—	—	—	—	—	—
<i>P. apicalifrons</i>	—	—	—	—	—	—	—	—	—
<i>P. apicalifrons</i>	—	—	—	—	—	—	—	—	—
<i>P. equidens</i>	—	—	—	—	—	—	—	—	—
<i>P. equidens</i>	—	—	—	—	—	—	—	—	—
<i>P. africana</i>	—	—	—	—	—	—	—	—	—
<i>P. africana</i>	—	—	—	—	—	—	—	—	—
<i>P. africana</i>	—	—	—	—	—	—	—	—	—
<i>P. typ.</i>	—	—	—	—	—	—	—	—	—
Total	80	7	3	3	83	46	106	47	77

TABLE I—continued

Species	Heiban, 18 12 42, in house by day	T-lodi, 6 11 40, house by day	Kassala, 15 2 42, in house by day	Kassala, 25- 27 3 43, traps in garden	El Fasher, 6 11 38, houses	Akobo, 5 1 40, in tent at night	Hawata, 15- 16 12 42 traps on ground	Hawata, 18 12 42 near light in stand- ing railway coach
<i>P. papatau</i>	—	—	1	3	48	—	—	—
<i>P. langeroni</i> var <i>orientalis</i>	—	—	—	13	2	12	27	8
<i>P. elydi</i>	—	—	—	58	132	31	22	11
<i>P. squamipennis</i>	—	—	20	30	3	5	3	3
<i>P. signatipennis</i>	42	40	2	13	—	—	1	5
<i>P. congolensis</i> var <i>distinctus</i>	2	21	—	11*	4†	—	—	—
<i>P. africanus</i> and var <i>sudanicus</i>	4	—	2*	—	—	—	—	—
<i>P. schuetzi</i> and var <i>aethiopicus</i>	—	—	—	—	—	—	—	—
<i>P. spp</i>	—	—	—	—	—	—	—	—
Total	18	79	34	128	189	52	53	30

+ 1 *P. adleri* 3 *P. affinis*• *P. p* var *bergeroti*

TABLE I—continued

Species	Hawata, 4-2 12 12 in net trap baited with man	Damasin, 28 10 39, in houses by day	El Hamra 19, 20 3 40 ground traps	Kosti, 1939, 1040, in buildings	Palore, 21 12 39, mosquito net, morning	El Obeid, 8 9 39, in houses	Yei, 1 2 45, traps on ground and in houses	Total
<i>P. papatau</i>	♂	0	4	131	♂	7	—	212
<i>P. langeroni</i> var <i>orientalis</i>	♀ Gorged	—	3	—	—	—	—	101
<i>P. elydi</i>	8	79	72	2	1	5	—	781
<i>P. squamipennis</i>	17	35	—	—	42	—	—	266
<i>P. signatipennis</i>	—	—	13	0	1	16	—	755
<i>P. congolensis</i> var <i>distinctus</i>	—	—	—	—	3	2	7	30
<i>P. africanus</i> and var <i>sudanicus</i>	—	—	7	1	—	53	—	450
<i>P. schuetzi</i> and var <i>aethiopicus</i>	—	—	4	—	—	1	2	249
<i>P. spp</i>	—	—	—	—	—	4*	43†	64
Total	60	605	114	143	2	118	52	2,908

• *P. adleri* + *P. afr* var *niger*

latrine, the close association already mentioned between lizards and *P. signatus* was evident, and this species could easily be caught in hundreds. We have on occasion spent the time dissecting large numbers of them, in the hope of finding the flagellate stages of lizard leishmaniasis, but without success. The lizards were never examined as this requires the use of culture media and other methods which were not available in the circumstances.

#### SANDFLIES AND THE GROUND

Sandflies are delicate insects unsuited to life in the conditions of high temperature and low relative humidity which are so evident by day in the Sudan plains during the dry season. TAYLOR (1938) showed that at 30° C and a relative humidity of 40 per cent. the mean length of life of fed females of *P. papatasi* (which, according to our records of distribution in the Sudan, is apparently one of the most xerophilic species of *Phlebotomus*) was 3.5 days, while the thermal death point in a 1 hour exposure was 41° C. At Wad Medani which is in the centre of the Gezira clay plain in the Sudan, the monthly mean temperature is above 30° C. from April to June, and the monthly mean relative humidity (from readings taken three times daily) is from 18 to 44 per cent. (*Climatological Normals*, 1938). Shade maximum temperatures exceeding 41° C have been recorded in 10 out of the 12 months of the year and the shade temperature has been known to reach 48° C (118° F). The mean shade maximum is more than 41° C in April and May.

The figures quoted for Wad Medani have been given, not because it is the hottest sandfly infested place in the Sudan, but because it is the principal centre in the area south of Khartoum in which we have collected extensively and accurate records have been kept there over a period of many years. Conditions in Wad Medani are modified to some extent by the continued cultivation which has been made possible there during the last 20 years as the result of irrigation started in 1925. We have collected *Phlebotomus* in other localities where during the period of our visit, conditions appeared less favourable than those in Wad Medani, but comparable meteorological data are not available for those places.

Conditions in the plains of the central Sudan are, in general similar to those described by BUXTON (1923) as occurring in desert areas—high temperature and bright sunlight by day with extreme diurnal range of temperature and relative humidity the latter varying inversely with the temperature. During the day when the temperature is high, the relative humidity may reach very low values (figures as low as 5 per cent. are occasionally recorded in Khartoum) but with the great fall in temperature which occurs at night the relative humidity increases to such an extent that dew may form, even in the desert (cf BUXTON p. 81). By day conditions appear impossible for sandflies. The sun-baked earth is burning hot and bare with no trace of greenery. The only vegetation evident in most areas is thin thorn scrub which at this time of the year appears

withered, leafless and dead. Although even shade temperatures are probably inimical to sandflies, no natural shade can be seen. The nearest water supply may be several miles distant. Yet at this time of year sandflies can often be found in abundance in such places by the methods we have described and the traveller who goes to bed without a sandfly net may have a disturbed night.

Generally, however, the sandflies appear only in the evening and at night when the temperature is low and the relative humidity in consequence is high. In other words, they appear, sometimes in large numbers, only at the time of day when physical conditions are suitable for their existence, and disappear before morning comes. It still has to be explained how they survive during the rest of the 24 hours, when conditions are so impossible. It is easy to postulate that they find suitable microclimatic conditions in shady places, rock crevices, tree holes, etc., which remain damp throughout the dry season, such as have been found elsewhere in connection with mosquito breeding, but in the places to which we refer no such microclimatic pockets can be found. In some of them no water can, in fact, be found for miles, and mosquitoes cease to breed during the dry season.

From observations and collections made by the methods described we have reached the conclusion that the colonization of such places by sandflies is due in large measure to their exploitation of a vast subterranean environment consisting of holes and cracks in the ground.

We recorded in our previous paper (KIRK and LEWIS, 1940) that sandflies can be captured readily from animal burrows by the use of oiled paper traps. We suspected then that there might be some specific relationship between certain sandflies and the burrows of particular animals. Further observations did not confirm this supposition, while we often obtained sandflies by the same methods from holes and cracks in the ground which were not inhabited by burrowing animals. It seems more likely, therefore, that the sandflies inhabit animal burrows merely because they find there physical conditions suitable for their existence. An account of the physical conditions in animal burrows can be found in BUXTON's (1923) book, *Animal Life in Deserts*, so it is not necessary to elaborate the details here. BUXTON has shown that it is only the surface layer of the soil which is liable to the great fluctuations of temperature and humidity characteristic of desert areas. At 6 inches depth the daily fluctuation is only about one-quarter of that in the surface layers, while at 10 inches it is only about one-thirtieth (less than  $1^{\circ}\text{C}$ ) and the relative humidity has a constant value of approximately 100 per cent. Therefore, by burrowing, an animal can easily reach equable conditions of temperature ( $27^{\circ}\text{C}$ ) and humidity (100 per cent)—approximately the optimum values for sandflies. The figures quoted may be taken as typical of results which have been obtained in desert areas in widely separated parts of the world, and observations in the Sudan show that conditions there are no different from those which have been found in other places. Referring specifically to *Phlebotomus*, VLASOV (1932) and



PETRISCHEWA (1935) have shown that in the plains of Turkmenistan animal burrows provide a suitable environment for sandfly breeding throughout the year when conditions above ground are impossible for the greater part of the year.

In the Sudan plains during the dry season there exists in addition to animal burrows an extensive system of cracks in the dark grey heavy cracking clay which is often called black cotton soil. These cracks are exceedingly numerous, often deep, and form an extensive network. The soil surface lining the cracks has a greater area than that above ground, so that one might visualize the cracks as constituting an extensive environment wherein rise pillars of earth, the tops of which constitute the visible surface of the ground (Fig. 1). We carried out some observations on physical conditions in these



FIG. 1.—Surface of cotton soil, showing the typical cracks.

cracks but after a few preliminary studies it occurred to us that the data we sought were probably well known to the soil-chemists of the Agriculture Research Division, and that their conclusions would be the result of observations extending over many years. So we addressed an enquiry to Dr H. GREENE, Chief Soil Chemist, who replied, 'Yes in cracked clay soil of the Sudan temperature at 2 feet depth is fairly steady at 37° C. and air within small cracks is practically saturated with moisture. In larger cracks air will be drier owing to convection currents which mix soil air with atmospheric air. GREENE (1937) wrote,

The rate of water loss from (Gozara) soil decreases with depth. At a depth of 2 or 3 feet the soil retains throughout the year sufficient moisture to saturat

any air present in the interstices" BUXTON (1936) showed how in Nigeria a clay soil with only a low water content could greatly affect the atmosphere in its spaces which reached a point near saturation

The plains of the central Sudan are composed largely of cotton soil, but scattered in the cotton soil, like islands in a sea, are areas of reddish yellow sandy soil of varying size—the so-called "azaza" grounds. The cotton soil is darker in colour than the azaza soil and typically clayey, so that it cracks in the dry season, and in the rains rapidly becomes waterlogged. Animals which make burrows in the cotton soil are therefore liable in the rains to be flooded out and drowned. In the more permeable azaza soil the chances of survival



FIG 2—Close-up photograph of cotton soil cracks. A tobacco pipe is included to indicate the scale.

during the rains are greater owing to better drainage, and one of the most noticeable differences between the azaza patches and the cotton soil is the greater number of animal burrows in the former.

There exists, then, an underground environment suitable for sandflies in the Sudan plains, even in the dry season, consisting of (1) animal burrows, more frequent in the azaza soil, and (2) cracks in the cotton soil forming a continuous, vast, subterranean environment which is largely obliterated during the rains. There is also considerable evidence that sandflies actually use this environment. We have seen sandflies coming out of the holes in the ground, and we have several records supplied by others who have collected *Phlebotomus* in the Sudan that the adults were seen emerging from cracks in the soil. The

principal evidence however is derived from collections made by the methods of capture we have described. We have seldom failed to catch sandflies in oiled paper traps left overnight at the mouths of cotton soil cracks, even when they could not be obtained by other means. We have found almost invariably that large numbers of sandflies can be caught by leaving oiled paper traps overnight at the mouths of animal burrows. The striking success of the latter method is probably due to the fact that the mouth of the burrow is a bottle-neck, through which all sandflies entering or leaving have to pass. The fact that sandflies are so frequently caught by traps in the cotton soil cracks, where there is no such bottle neck and their chances of flying in and out without coming against the trap are very great, indicates that the sandfly population in those cracks must be considerable. Finally sandflies often appear at night in great numbers in places where no possible diurnal environment exists above ground, and one can only conclude that they come from this subterranean environment. To test this conclusion we have on one or two occasions erected traps made of sandfly netting with a caged animal inside, the traps being devised in such a way that the only possible way of entry was from the cracks in the ground inside the net. By suitable methods, such as visiting the traps at various times during the night we were generally able to find sandflies in the traps, in spite of the fact that they had very good chances of disappearing back into the ground before being caught. Sandflies are probably not the only inhabitants of this underground environment other insects, scorpions and lizards also appear to live in it. When a piece of cracked clay is flooded for irrigation crickets may be seen emerging from the cracks as the water pours in. In the Gezira the invasion of houses by scorpions driven out of their cracks by the first heavy rains, and the great diminution of sandflies at this time, are well known phenomena. Near Wad Medani in 1945 when the rains were about a month late a resident reported that by the beginning of September scorpions had given little trouble but that sandflies had been much worse than usual and had only just begun to diminish, a month after the usual time.

We have no direct observations to show whether the sandflies actually breed in these cracks and holes in the ground, or only use them as resting places in the adult stage. We have not made any thorough search for the immature stages as these are extremely difficult to find in nature. KING (1913) however found one larva of *Phlebotomus* in the cotton soil at Tokar at the depth of about 4 inches and later (1914) larvae and pupae in earth in Khartoum, so it is probable that they do breed in these situations. Conditions in animal burrows are suitable for sandfly breeding throughout the year but we do not know what happens to the sandflies in the cracked cotton soil when their environment is obliterated by the rains. We do not know whether they get over this difficulty by a period of hibernation such as has been observed in some Palearctic species, whether some survive in a few remaining cracks, or whether the cotton soil cracks, as they open again in the dry season are re-colonized from the arazi grounds.

This close association between sandflies and the ground is not without interest in the epidemiology of kala-azar. The fact that the infection tends to cling to certain sites was emphasized many years ago by DODDS-PRICE and ROGERS (1914), who wrote, "Were it not for the facts known regarding the life history of the parasite, the evidence would go far towards incriminating the actual soil as well as the houses." The facts regarding the life history of the parasite to which these authors refer were PATTON's observations on the development of leishmania in the bed bug. DODDS-PRICE and ROGERS found that even burning down of the houses so that only the four walls remained and rebuilding on the same site did not stamp out the disease, infection of the actual site being said to last as long as a year. In the Sudan it is said that even ground which has been uninhabited for some years may be infected, since cases immediately occur if it is re-occupied (ATKEY, 1931). We have never been able to verify this personally, but there is nothing improbable in the supposition that it may occur if the sandflies persist in the ground as we have described. We have found cracks and holes in the ground the best places to capture most of the species occurring in the Sudan plains, including *P. langeroni* var *orientalis*, which has been found in quite uninhabited country. Persistence of the infection in an animal reservoir, or the inclusion of an individual with post-kala-azar dermal leishmaniasis among those attempting to occupy an uninhabited area where the vector was present might rapidly lead to the occurrence of fresh cases.

#### SUMMARY

1 Twenty-five named species and varieties of *Phlebotomus* are recorded from the Anglo-Egyptian Sudan, and the methods which have been used in collecting are described.

2 Observations are recorded on distribution, bionomics, colouration, feeding habits and other matters.

3 Flagellates undergoing "anterior" development have been found in specimens of *P. langeroni* var *orientalis* which had fed on a case of post-kala-azar dermal leishmaniasis.

4 A vast subterranean environment is described which exists in the Sudan plains and is constituted by holes and cracks in the ground. It is suggested that this is the main habitat of sandflies in the wilderness.

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## CANINE VISCERAL LEISHMANIASIS IN VILLAGES WEST OF LANCHOW, CHINA

BY

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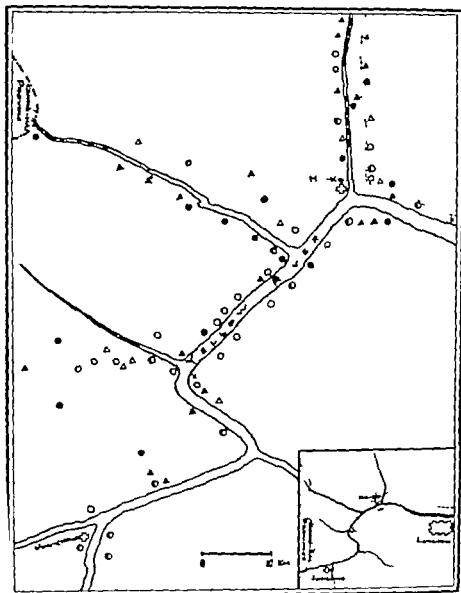
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The idea that the dog is the reservoir host of kala-azar originated from the discovery by NICOLLE and COMPTE of canine visceral leishmaniasis in Tunis in 1908. The investigations of many workers along this line indicate that the disease is prevalent in the Mediterranean region, while in India, where kala-azar is endemic, its existence is considered either very rare or entirely absent. The recognition of the disease in China is only a recent development. In 1934 ANDREWS first reported a naturally infected dog from Mukden, LEE (1937) found another two in Peiping, and in 1939 it was proved beyond doubt that the infection was common among dogs in the latter city (FENG, CHUNG and HOEPPLI, 1939, HOEPPLI, 1940, CHUNG, 1940, Ho, YUAN and CHU, in press). CLOW (1943) has reported one case from Sian. This communication records forty-four proven cases of canine leishmaniasis occurring in villages west of Lanchow.

### AREA UNDER STUDY

The area under study covers approximately 60 sq km of mountainous region and includes the major portion of Jung-ching Hsien, the western part of Ko-lan Hsien and the southern tip of Jung-teng Hsien. It is about 50 km west of Lanchow, adjacent to the east border of Ching-hai Province. The whole area is watered by the Yellow River and its branches (see Map). Scattered



*Explanation of markings*

- = village with canine leishmaniasis
- = village with human kala-azar
- △ = village with both human & canine visceral leishmaniasis
- × = village in which cases of human & canine visceral leishmaniasis have not been found
- = village not examined

on the river banks are small villages and hamlets. The soil is loose and alluvial and the altitude ranges from 5,656 to 7,788 feet above the sea level.

### METHOD OF STUDY

The method employed in the present investigation is based upon the one used by HO and YUAN with slight modification. Villages and hamlets are visited and all their dogs inspected for gross skin lesions. Suspicious dogs are then picked out and examined for evidence of leishmaniasis by means of skin biopsy and ilium puncture (HO, CHU and YUAN, 1940 and 1944).

Members of the family living in the households in which infected dogs were found are examined for clinical evidence of kala-azar. The past family history concerning possible existence of the disease is recorded.

### RESULTS

During a period of 6 months, from August, 1944, to February, 1945, 104 small villages and hamlets with altogether 5,970 households were visited, and 1,430 native dogs inspected for gross skin lesions. One hundred and ten dogs with suspicious signs or lesions were picked out and examined for evidence of leishmaniasis by means of skin biopsy and ilium puncture. Among them forty-four dogs from thirty villages were found to have visceral leishmaniasis. Of the forty-four naturally infected dogs, twenty-five were encountered in kala-azar endemic villages and the remaining nineteen in villages in which no kala-azar case was proved to exist. In the following two instances, coincidences of both canine and human visceral leishmaniasis in the same households were observed.

#### Case 1

A native male dog 6 years old (Dog 19) began to have whitish scaly lesions over face and ears in 1942. As time went on, these lesions became sometimes worse and sometimes better. Examination on 30th August, 1944, revealed that the dog had bilateral blepharitis and cutaneous lesions characterized by seborrhoea and epilation over the above-mentioned parts. His general condition was robust. Skin biopsy and ilium puncture were positive for leishmania.

Next door to this dog was Dog 20. (In the same household with the latter animal there had been another adult dog, which had suffered from similar skin lesions for 2 years and died in the middle of August, 1944.)

Living in the same household with this dog were five adults, two children and an infant of 3 months old. All of them were apparently healthy and normal when the dog was first discovered in August. Four months later, e.g. in December, the infant began to have irregular feverishness and was seen in our kala-azar clinic on 8th April, 1945. Physical examination showed that the patient was under-nourished and under-developed, very anemic and critically ill. Rectal temperature, 37.6° C. Moist rales were present over both lung



bases. Heart normal. Spleen was not palpable and liver 5 cm. below costal margin. Urine was negative for albumin. R.B.C. 2,250,000 Hb 30 per cent. and W.B.C. 5,600 Globulin test was positive. Sternal puncture revealed many leishmania. The patient died in spite of treatment on 28th April, 1945.

### Case 2.

A native male dog, 6 years old (Dog 15) was noticed to have epilation for 3 years. Examination on 30th September 1944, showed that the dog had slight generalized epilation and seborrhoea which were more conspicuous over face and ears. Illum. puncture was positive for leishmania. Skin biopsy negative.

Living in the same household were a child 1½ years old and his parents. The parents were apparently normal while the child was found to have anaemia and splenomegaly. The parents said that the child had suffered from irregular feverishness, occasional cough and progressive pallor for more than half a year. The child was seen in our clinic on 16th October 1944. Physical examination revealed that the patient was under nourished and under-developed. Weight, 17 lb Rectal temperature, 36.8° C. Spleen and liver were 10.5 and 3 cm. below costal margin respectively. R.B.C. 1,400,000, Hb 34 per cent. and W.B.C., 3,950 Globulin test was strongly positive and sternal puncture revealed many leishmania. The patient apparently recovered after a course of urea stibamine. Follow up examination on 5th March, 1945 showed that the child was apparently healthy and normal. Weight had increased to 23 lb. Spleen remained only 3 cm. below the costal margin and liver was not palpable. R.B.C. 5,100,000, Hb 80 per cent. and W.B.C. 8,400 Globulin test was weakly positive and sternal puncture negative for leishmania.

While the disease was apparently absent in the majority of the villages under study its incidence in some was high. In twenty-seven villages the infection was detectable in one out of every twelve dogs inspected for gross skin lesions and in another three hamlets the infection rate was even higher. For instance, three out of seven dogs in Wang-chia-ho-tam (7,788 feet above sea level) four out of thirteen in Fu-tze-chung (5,742 feet above sea level) and two out of eleven in Huan-tu-tan tze (5,742 feet above sea level) were found to have the infection with dermal manifestations. For the whole area under study the infection rate is estimated to be at the minimum 3 per cent. and the distribution of the disease is widely spread. (See Map, page 890.)

The clinical picture of naturally infected dogs is well known to the natives. A large majority of their so-called ugly dogs were found by us to be infected with leishmaniasis. Cutaneous lesions characterized by epilation and seborrhoea were present in all but one proven case, and were usually more conspicuous over face and ears, resembling unca lesions of the human scalp, and always bilateral in distribution. The hair of the infected dogs appeared coarse and lustreless but their general condition, in the majority of cases, was apparently robust. Bilateral blepharitis was seen in eight cases and symptoms

such as anorexia and sluggishness observed in only a few. Three-fourths of the infected dogs had reached an approximate age of 5 years or over. None were under 2 years old.

### DISCUSSION

Leishmania infection in the dog, as in man, manifests itself either as pure cutaneous leishmaniasis or as visceral leishmaniasis with or without dermal manifestation. In China only the latter form of infection has been so far reported. Based upon the clinical pictures and the presence of leishmania in the bone marrow, it is concluded that the forty-four cases described in this communication are of visceral leishmaniasis with dermal manifestation.

The fact that naturally infected dogs are so well known to the natives indicates that canine leishmaniasis has probably been endemic in North-West China for a considerable period. In Peiping, North China, the condition was not so clear-cut. Firstly, the general public in Peiping was not well acquainted with this canine disease, and secondly, according to HO, YUAN and CHU, most of the naturally infected dogs there were of foreign breed. Therefore it is still questionable whether the disease there is newly introduced from outside or whether it has long existed without being recognized by the general public as well as by medical men.

It is generally held that kala-azar rarely occurs above an altitude of 4,000 feet (HO and his co-workers, STRONG, 1943). The same is probably true with canine leishmaniasis. In the investigation conducted by the authors, it was, however, found that canine visceral leishmaniasis, as well as human kala-azar, not only does occur but also becomes endemic in an area with an altitude ranging from 5,656 to 7,788 feet above the sea level.

That both canine and human visceral leishmaniasis are equally prevalent in villages west of Lanchow is an established fact. This phenomenon was not observed in the rural district adjacent to Peiping, where, according to HO, YUAN and CHU, continuous examination of dogs during a period of 40 months revealed only four naturally infected among 1,780 examined from three of forty-five kala-azar endemic villages. It seems that more investigations should be made in other kala-azar endemic foci before a clearer epidemiological picture of canine leishmaniasis in relation to kala-azar in China as a whole can be described.

### SUMMARY

Forty-four dogs naturally infected with visceral leishmaniasis were found among 1,430 examined in villages about 50 km west of Lanchow. Epilation and seborrhoea were present in all but one of the dogs. Leishmania were found by skin biopsy in thirty-eight of the forty-four dogs and by ilium puncture in thirty-nine of forty-one dogs in which this operation was performed. The altitude of the area under study ranges from 5,656 to 7,788 feet above sea level. On this high plateau kala-azar, as well as sandflies, was also found to be present.

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# LEPTOSPIRA ICTERHAEMORRHAGIAE IN RATS OF BEIRUT

BY

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The present investigation was carried out shortly after two cases of Weil's disease were discovered in the hospital of the American University of Beirut. The aim of this survey was (a) to determine the incidence of leptospirosis in the wild rats of Beirut, (b) to evaluate the various methods of examination used for this purpose, and (c) to study the kidney sections, specially for possible pathological changes produced by leptospirae. During these examinations certain observations were made, which though unrelated to leptospirae, are included in this report.

## PROCEDURE

Seventy wild rats were trapped alive in Beirut during the period from 21st May to 8th October, 1945, of which sixty-five were identified as *Rattus norvegicus* and five as *R. alexandrinus*. They were caught in houses, shops, stables and streets in different sections of the city. They were killed in the laboratory and immediately examined as follows. Both kidneys were removed under aseptic precautions through incisions in the flanks. One kidney was

cut in two halves and fixed in 10 per cent. formalin, one half for silver impregnation according to NYKA's (1934) modification of the Levaditi method, and the other half for haematoxylin-eosin stain. The second kidney was placed in a sterile mortar and emulsified in about 5 c.c. of sterile saline. One drop of this emulsion was examined under dark field illumination and, if found positive or suspicious for leptospirae, cultures were made in Schöffner's buffered medium (LEWIS 1942) and 2 c.c. were inoculated intraperitoneally into young guinea-pigs weighing 200 to 250 grammes. The cultures were examined daily after 72 hours of incubation at 34 C. The guinea-pigs were observed for evidence of sickness particularly rise of temperature and appearance of jaundice. The infected guinea-pigs either died 5 to 10 days after inoculation or were killed around the 10th day and autopsied immediately. The characteristic postmortem findings of haemorrhages in the subcutaneous tissues, kidneys, lungs, gastro-intestinal tract and elsewhere were noted. Kidney and liver emulsions were examined under dark field illumination and cultured, and portions of the liver and kidneys were fixed in 10 per cent. formalin for silver impregnation.

Every rat was not examined by all the methods described. Dark field examination of the kidney emulsion, and examination of at least one kidney section impregnated with silver and one section stained with haematoxylin-eosin, were carried out in all cases. Guinea-pig inoculation and cultures were made only when dark field examination of the kidney emulsion gave a positive or doubtful result.

## RESULTS.

### A. LEPTOSPIRAE.

Leptospirae were found in eight of seventy rats of all sizes (11.4 per cent.) in seven of twenty rats above 20 cm. (35 per cent.) and in one of fifty rats under 20 cm. (2 per cent.). All positive rats were *R. norvegicus*.

#### *Results and reliability of the individual methods of examination.*

Leptospirae were seen in the silver impregnated kidney sections of each of the eight positive rats. As there were no cases where leptospirae were detected by other methods but not seen in the kidney sections, this method of examination was considered the most reliable one realizing at the same time the possibility that even by this method other positive cases might have been missed.

Examination of the kidney emulsion under dark field illumination gave the next highest number of positive results. Six of the eight positive cases were detected by this method (75 per cent. reliable as compared with the former method), and in the seventh case (Rat 36) atypical structures resembling leptospirae were seen.

By inoculation of the kidney emulsion into young guinea-pigs, five of the seven positive cases examined could be detected (71.4 per cent. positive).

Culture of the kidney emulsion gave the least number of positive results, as only four of the seven positive cases examined were detected by this method (57.1 per cent positive)

*Trypanosoma lewisi*, the common haemoflagellate of rats, was detected in the haemorrhagic kidney emulsion examined under dark-field illumination, in eight of the seventy rats (11.4 per cent). All positive rats were under 20 cm in length. Therefore, according to this method of examination, the incidence of trypanosomiasis in fifty rats under 20 cm was 16 per cent. Seven of the positive rats were *R. norvegicus* and one was *R. alexandrinus*.

#### *Examination of the kidney sections impregnated with silver*

The leptospirae were located on the surface of the epithelial cells of the convoluted tubules, often in great numbers, forming masses that filled the lumen and made detection easy. The number of parasitized tubules varied greatly from case to case and in different sections of the same kidney. In one positive kidney where infection was heaviest (Rat 69), masses of leptospirae were seen in the lumen of some of the collecting tubules forming casts, moreover, one or more leptospirae were also seen occasionally in the connective tissue around these tubules. In another case (Rat 14), where leptospirae were found in the kidney emulsion—by dark-field examination, culture, and guinea-pig inoculation—they were not seen in multiple sections made from one-half of the second kidney, but found in sections from a portion of the other half of the same kidney.

#### B HISTOLOGICAL EXAMINATION OF KIDNEY SECTIONS STAINED WITH HAEMATOXYLIN-EOSIN

In most sections there was a mild degree of cloudy swelling, with a little amount of albuminous fluid in the convoluted tubules and an occasional hyaline cast in the collecting tubules. In the absence of other pathological changes, these findings were not considered significant.

The significant positive findings were —

1 *Nephritis*—Histological changes similar to acute or sub-acute glomerulonephritis of man, were seen in the kidneys of fourteen of seventy rats. The glomerular changes consisted of occasional polymorphonuclear leucocytes in the tuft, hyaline thickening of the wall of the capillaries with resulting ischaemia, albuminous fluid in the capsular space and adhesion of the tuft to the parietal layer of Bowman's capsule. The tubular changes consisted of albuminous degeneration of the epithelial cells of the convoluted tubules, with the formation of hyaline drops in several cases, vacuolation of these cells in some, and necrosis of tubular epithelium in other cases. There were foci of small round cells in the interstitial connective tissue. The arterioles showed hyaline thickening of their wall with narrowing of the lumen. The parietal layer of Bowman's capsule was little or not affected, and there was no fibrosis of the kidney parenchyma.

Nephritis was observed only in the older rats (above 18 cm. in length). Six of the fourteen rats with nephritis were positive also for leptospirae.

2. *Pyelitis*.—Two cases of pyelitis were seen (Rats 15 and 24) with many eosinophils and polymorphonuclear leucocytes in the pelvic connective tissue. One of these kidneys (Rat 24) harboured leptospirae and showed glomerulonephritis. In both kidneys, the nematode *Trichosomoides crassicauda* (*vide infra*) was found in the pelvis, and was apparently responsible for the pyelitis.

3. *Brown pigment*.—Varying amounts of brown pigment were seen in the epithelial cells of the convoluted tubules in twenty five kidneys, but it was abundant only in eleven of them. The pigment appeared as small brown granules in the cytoplasm of the epithelial cells, but brown homogeneous globules larger than the nuclei of these cells were also seen in several cases. According to the histochemical methods used (Prussian-blue reaction and sudan III applied to frozen sections), iron and lipoids could not be demonstrated in the pigment, which appeared to be bilirubin.

Of the twenty five kidneys showing pigment, twenty-one were those of older rats (above 18 cm.). Each of the eight kidneys positive for leptospirae showed pigment, and in seven of these the pigment was abundant. Each of the fourteen kidneys with nephritis contained the pigment, and in nine of these the pigment was abundant.

4. *Trichosomoides crassicauda*.—This nematode, commonly found in the urinary passages of rats, and described in detail by THOMAS (1924), was discovered by chance in sections in the kidney pelvis of three rats. Owing to this unsatisfactory method of examination no conclusions could be drawn concerning its incidence in the seventy rats examined. According to the cross-sections studied, one was the larval form imbedded in the epithelium of the pelvis (Rat 15) the second was the small adult male, free in the pelvis (Rat 18), and the third was the much larger adult female free in the pelvis (Rat 24). The co-existence of a pyelitis in Rats 15 and 24 has already been mentioned.

5. *Leptospirae*.—Although individual leptospirae are not demonstrable with haematoxylin-eosin stain, yet where the micro-organisms were clumped together in great numbers filling the lumen, this mass appeared faintly bluish, not unlike mucoid, yet quite distinct from the pinkish albuminous precipitate seen in the tubules. No histological difference could be noted between the epithelial cells of the convoluted tubules infected with leptospirae, and the epithelial cells of the convoluted tubules not harbouring leptospirae.

#### DISCUSSION

It has been shown by SCHÖFFNER and KUTJEN, and other Dutch workers quoted by SCHÖFFNER (1934) and WALCH-SCHÖDINGER (1939), that the incidence of leptospirosis is distinctly higher in older rats. The investigations of LEWIS (1942) and LARSON (1943), as well as the results of the present survey confirm this finding.

In evaluating the reliability of the different methods used to detect leptospirosis of rats, accurate conclusions cannot be drawn unless all the methods are applied to each case and an adequate number of cases are studied. However, judging from the results of different surveys, it appears that demonstration of leptospirae in kidney sections impregnated with silver, and detection of immune bodies in the blood serum, are the two most reliable and complementary methods. Examination of the kidney emulsion by dark-field illumination, guineapig inoculation and culture, are comparatively less reliable, yet if conducted carefully, they will detect a high proportion of the positive cases. If an infected rat remains a carrier of leptospirae for life, then the serological tests will be highly reliable, but if the infected animal ceases to be a carrier, the immune bodies will persist in the blood serum for a time, and the serological tests may consequently give results higher than the actual incidence of infection. However, in all the cases that LARSON found to be positive by this method, leptospirae could be demonstrated by one or more methods.

Unlike that of leptospirosis, the incidence of rat trypanosomiasis is distinctly higher in *young* rats, as shown in the present survey and as reported previously by HERRICK and CROSS (1936) and DUCA (1939).

It is probable that the brown pigment encountered in the sections was deposited in previously damaged epithelial cells. The association of marked albuminous degeneration with heavy pigment deposition lends support to this view. Moreover, the ischaemia resulting from the vascular changes seen frequently in the old rats with or without nephritis, may have been responsible for the tubular degeneration.

#### SUMMARY

The kidneys of seventy wild rats caught in Beirut were examined for leptospirae and eight were found to be positive. The incidence was 2 per cent in fifty young rats under 20 cm, and 35 per cent in twenty rats above 20 cm.

Of the four different methods used, examination of the kidney sections impregnated with silver gave the highest number of positive results. Examination of the kidney emulsion under dark-field illumination, inoculation of the kidney emulsion into young guineapigs, and culture of the kidney emulsion gave respectively fewer positive results.

*Trypanosoma lewisi* was found in eight of the seventy rats examined, all positive rats being under 20 cm in length.

The nematode, *Trichosomoides crassicauda*, was found in sections of the kidney pelvis of three rats, two of which showed an acute pyelitis.

Histological examination of the seventy kidneys revealed fourteen cases of glomerulo-nephritis. Abundant brown pigment was deposited in the epithelial cells of the convoluted tubules in eleven kidneys, seven of which were positive for leptospirae, nine for glomerulo-nephritis, and five for both.



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## THE SURVIVAL OF TRANSFUSED ERYTHROCYTES IN SICKLE-CELL ANAEMIA.

BY

A ALTMANN, MAJOR, S.A.M.C.\*

*From the Central Military Laboratory, S.A.M.C*

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The mechanism of haemolysis in acute and chronic haemolytic anaemias is still obscure. It is impossible to explain haemolysis in all haemolytic anaemias on the basis of a single factor, it appears that in some a haemolysin is the cause of blood destruction, whilst in others the erythrocytes are defective. DAMASHEK and his co-workers (1938, 1940, 1943) put forward the view that in haemolytic anaemias haemolysis is an active process, a circulating haemolysin damaging the R.B.C.s, spherocytosis and increased fragility to saline are indicators of damage to normal erythrocytes by such circulating haemolysin. These authors include nocturnal paroxysmal haemoglobinuria and familial haemolytic jaundice as "possibly" caused by the action of a haemolysin, whilst physical factors (heat, cold, change of pH), mechanical factors, stasis in the spleen and splenic dysfunction (hypersplenism) are only of secondary importance. They also widen the meaning of haemolysin so as to mean "any substance causing injury to the red cells" which then also includes "hereditary injury" to the red cells, as in Mediterranean anaemia (Cooley's) and sickle-cell anaemia. HAM (1939), on the other hand, considers paroxysmal nocturnal haemoglobinuria to be due to an abnormality of the red cells which are lysed under conditions of stasis and shift of pH. He supports this view by experiments, in which he showed that *in vitro* normal serum lysed cells of cases of

\*My thanks are due to Col E. H. CLIVER, Director of Pathology, S.A.M.C., for permission to publish this paper.

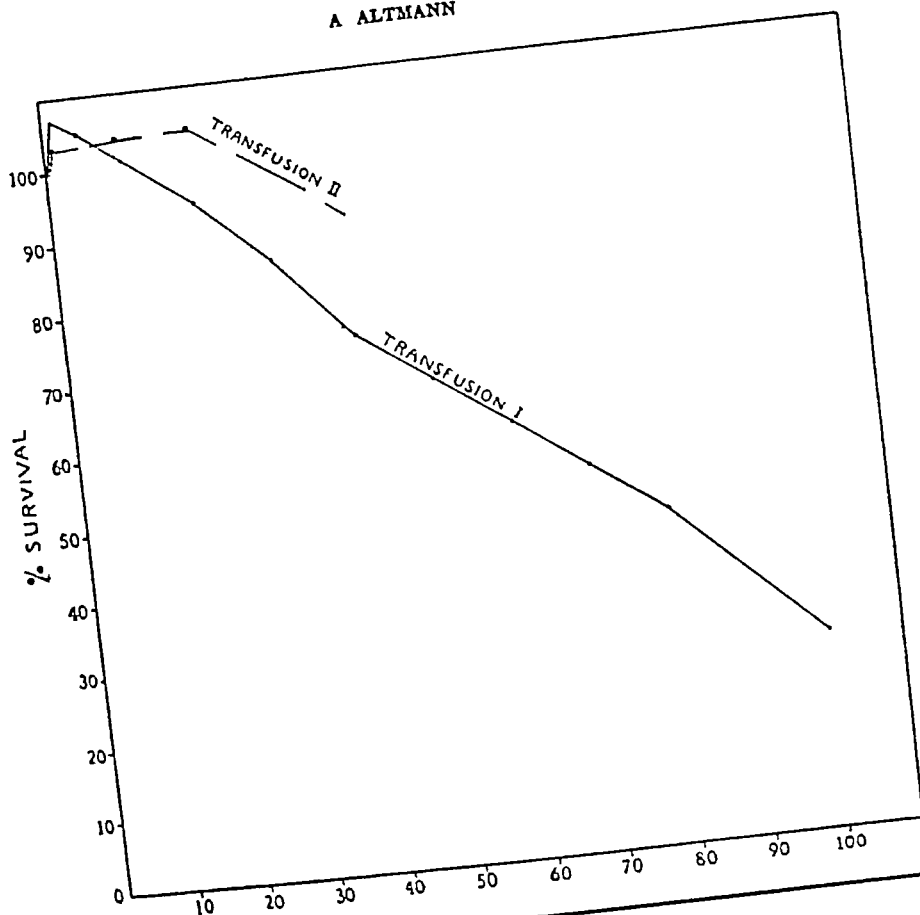
paroxysmal nocturnal haemoglobinuria, whilst serum of such patients did not affect normal cells. A failure to demonstrate haemolysins *in vitro* however is no proof of their absence in erythroblastosis foetalis, for instance, a haemolytic anaemia due to passive transfer of immune haemolysins from mother to child (WIENER, 1944; BORMAN DODD, MOLLISON, 1942) the presence of a haemolysin or agglutinin can rarely be detected in the serum of the infant. Using the survival time of transfused R.B.C.s as a criterion for presence or absence of a haemolysin, DACE and FIRTH (1943) showed that in paroxysmal nocturnal haemoglobinuria the patient's cells disappeared quickly when transfused into normals, whilst normal cells transfused into patients showed a normal survival time. From these facts DACE and FIRTH concluded that HAM's conception of this condition as a defect of the red cells is correct, and that haemolysis is not due to circulating haemolysins. Using the same technique, DACE and MOLLISON (1943), established the same fact in acholuric (familial) jaundice. Normal cells survive normally in such patients whilst the patient's cells are quickly eliminated from the circulation of normals therefore these authors concluded that in acholuric jaundice a defective erythrocyte, and not a circulating haemolysin, is the cause of haemolysis. In blackwater fever normal cells as well as the patient's cells are affected by the lysis process (FOR *et al.*, 1941) but blackwater fever cells are also eliminated quickly when transfused into normal individuals (FOR *et al.*, 1945) so it appears that in blackwater fever a toxin so damages normal cells that they are quickly lysed even in a normal medium.

In sickle-cell anaemia there undoubtedly exists a hereditary anomaly of the erythron. It is a haemolytic anaemia with reticulocytosis, raised icteric index, urobilinuria, and frequently with acute haemolytic crises. Recently a case of active sickle-cell anaemia in a South Africa born European was observed (ALTMANN 1945), and as transfusions were required therapeutically it seemed worth while to follow up the fate of the transfused cells.

**Method**—Concentrated R.B.C.s (about 750 c.c.) from Group "O" donors after not more than 24 hours storage were transfused into the patient (Group "A", Rh positive) as anticoagulant for the first transfusion 3.8 per cent. sodium citrate was used (10 c.c. per 100 c.c. blood) for the second transfusion a sodium citrate-citric acid-glucose mixture (LOOTTT and MOLLISON 1943). The inagglutinable count was determined before, immediately after the transfusion and at frequent intervals by the method of differential agglutination (MOLLISON and YOUNG, 1940; DACE and MOLLISON 1943).

**Results**.—The table and figure show the results of the transfusions. After both transfusions the disappearance curve is a straight line the slope of this curve is of much greater importance for judging the survival of transfused red cells than the endpoint (BROWN *et al.*, 1944). The linear course of the disappearance curve represents the normal rate of decay whilst any other haemolytic mechanism will produce a curved line of disappearance.

A. ALTMANN



	Percentage survival after (Days)											
	1	5	10	20	30	40	50	60	70	80	90	100
Transfusion I 26th, May 1944	107	105	101	94	85	74	67	60	53	46	36	28
Transfusion II 3rd, Jan 1945	103	—	104	98	90							

Inagglutinable count immediately after Transfusion I 767,000 (= 100 per cent)  
 Immediately after Transfusion II 845,000 (= 100 per cent)

On both occasions the transfusion was given when patient had a crisis as evidenced by fever, joint pains, raised icteric index and urobilinuria. The transfusions were well tolerated, no reaction occurred and the acute symptoms

subsided quickly. After the first transfusion the patient's blood count fell to the pre-transfusion level within 20 days after transfusion (4.2 ml. and 10 grammes Hb. maximum after transfusion, 5.2 ml. and 11.6 grammes).

This indicated that active blood destruction was taking place during this time and also later as the icteric index remained raised, and urobilin was present in the urine. But this destruction affected only the patient's own cells: the transfused cells disappearing normally at a constant rate of approximately 1 per cent. daily. Similar results were obtained by DACEY and FIRTH (1943), in paroxysmal nocturnal haemoglobinuria and in familial acholuric jaundice, proving normal survival of transfused R.B.C.s in such patients. BIRK and BULL (1943) found that in sickle-cell anaemia during a severe crisis, 85 per cent. of transfused cells were destroyed within 3 weeks following transfusion: that means an increased rate of destruction of transfused cells. But their findings are open to doubt, because these authors used the number of unsickled cells in a wet scaled specimen as index of the survival of transfused cells. Sickling, however, is an erratic phenomenon, not always easily reproducible to the same percentage and depending on temperature, white blood count, O tension and bacterial contamination (SHERMAN 1940). So a count of unsickled cells seems to be an unreliable method of counting surviving cells. In addition their patient had many transfusions and an increased elimination rate of the transfused blood due to anti Rh immunization was not excluded.

### SUMMARY

Normal cells transfused into a patient with active sickle-cell anaemia showed normal survival with a linear disappearance curve. The haemolytic process in sickle-cell anaemia only affects the patient's own red cells which are abnormal due to a hereditary factor whilst normal red cells remain unaffected.

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## A STUDY OF THE POSTMORTEM BONE MARROW FROM CHOLERA CASES

BY

H N CHATTERJEE,

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The present study is a continuation of previous observations by the writer (CHATTERJEE, 1939). It has been possible to secure the specimens of marrow from femur, humerus and tibia from a further fifteen cases, in addition to the twenty-five cases already studied. Both the transverse and longitudinal sections were studied from the different specimens.

### NAKED EYE APPEARANCE.

The bone marrow may present a red appearance in cases in which the reactive hyperplasia is marked. In others pink areas interspaced with white or fatty marrow present the usual picture. The general consistency is more solid than that of inactive fatty marrow and the specimens are sectioned more easily.

### MICROSCOPICAL EXAMINATION

The microscopical picture is characteristic. The most important feature of this is the great dilatation of the capillaries (Figs 1 and 2). In fact, this

disease presents a unique opportunity of studying the vascular bed of bone marrow particularly the collapsed capillaries of DOAN (1922 a and b) as well as the sinusoids (NEUMANN 1869) of the marrow

#### THE CAPILLARIES OF DOAN

These are greatly opened up and distended with red blood corpuscles and one can see continuous tracks of erythrocytes in the sections. The distension of the capillaries is seen, not only in bone marrow but also in many other different internal organs, *e.g.*, spleen, liver heart muscle, etc. (CHATTERJEE, 1939). But the brunt of the changes seems to fall greatly on the collapsed capillaries of the bone marrow and in no other organ is this distension so acute or so remarkable. In fact the diameter of these capillaries is seen to be dilated fifteen or twenty times, or even more. In the femur the tibia and the humerus the changes are essentially the same. Consequently it is worth while to evaluate the part which the extensive capillary dilatation of the marrow plays in the production of the extreme collapse and shock found in this disease after the passage of one or a few stools only. The total volume of bone marrow has been estimated by LUTWIG (1920) to be nearly 3 000 c.c. and 4 192 c.c. in a 20-year-old and a 55-year-old man respectively. Consequently this extensive marrow capillary dilatation in cholera naturally arrests our attention. On tracing these capillaries one can often see them open into the sinusoids which are also greatly dilated. These sinusoids therefore receive blood from the collapsed capillaries as well as from the regularly well formed capillaries, the openings of both varieties of capillaries being often demonstrable in the same identical sinusoid.

#### THE SINUSOIDS.

It is a common sight to see widely dilated sinusoids in the sections.

They are comparatively much wider channels lined by a single layer of endothelial cells—the latter being placed widely apart and connected to adjacent cells by silver staining reticular tissue. As has been already mentioned, it is possible to see the widely dilated collapsed capillaries as well as the well-formed capillaries both opening into the venous sinusoids.

#### OTHER CHANGES.

Another frequently observed change—the leucoblastic reaction which is fairly constant but is variable in degree. This is usually of a moderate degree the fat being never completely absorbed as is the case in acute septic infections such as meningitis—consequently a peculiar appearance is seen in the greater number of cholera cases. Columns of red cells occupy the centre—on either side of the red cells lie the leucoblastic cells one or more layers deep—all the structures lie within the thin-walled blood vessels—the collapsed capillaries as well as the sinusoids. It appears that the masses of red cells have come

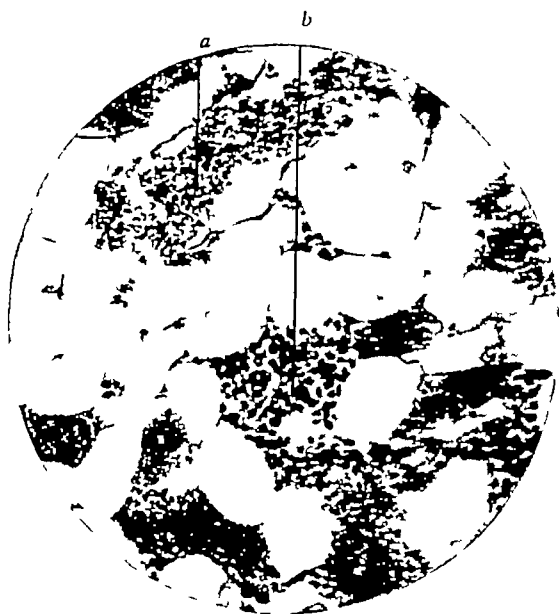


FIG 1—Bone marrow from the middle of the shaft of the femur from a case of cholera, showing great dilatation of the normally collapsed capillaries and marked congestion (a) There is also leucoblastic reaction in places (b)  $\times 215$  approx

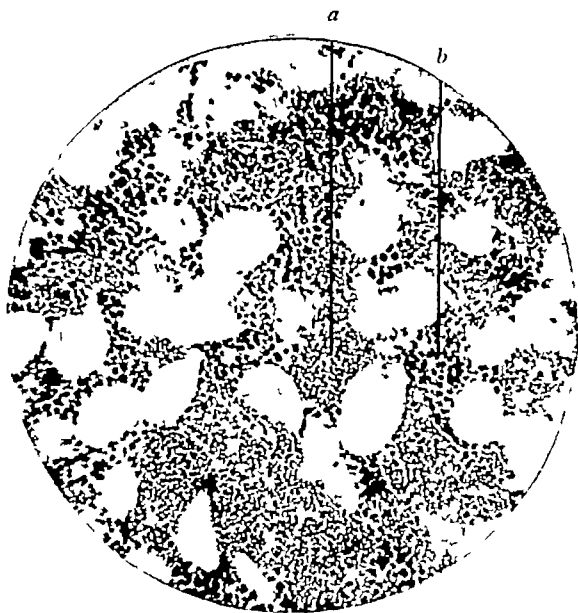


FIG 2—Bone marrow from the middle of the shaft of the femur from a case of cholera showing great dilatation of the normally collapsed capillaries and marked congestion (a) There is also leucoblastic reaction (b) in many places as can be noted by the dark nuclei  $\times 150$  approx

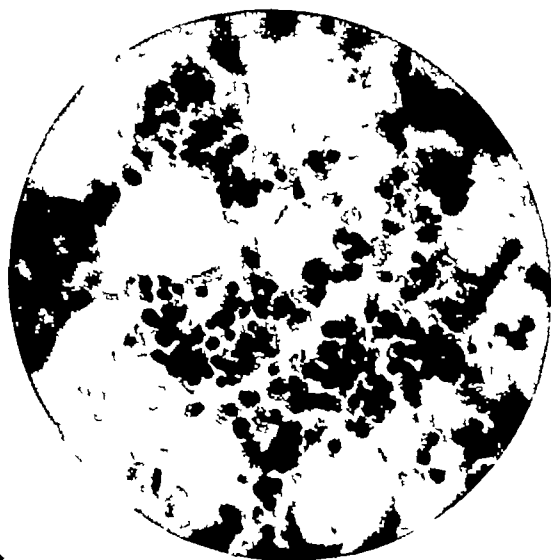


FIG 3—Bone marrow changes in cholera showing large number of eosinophil cells  $\times 700$  approx

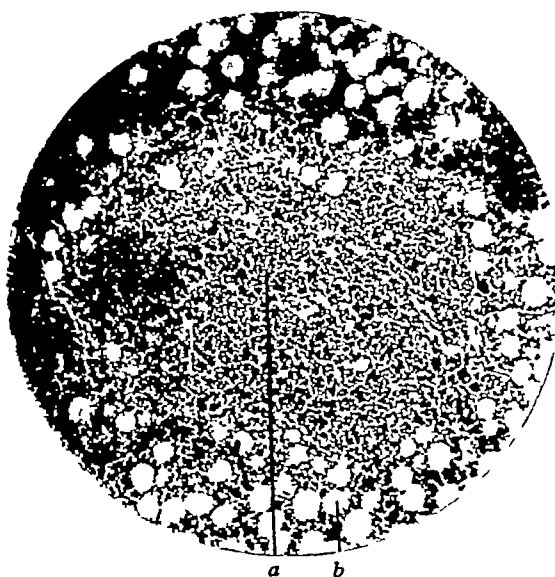


FIG 4—Section showing a lymphatic nodule in the bone marrow of a case of cholera (a) the usual bone marrow structure (b)  $\times 75$  approx



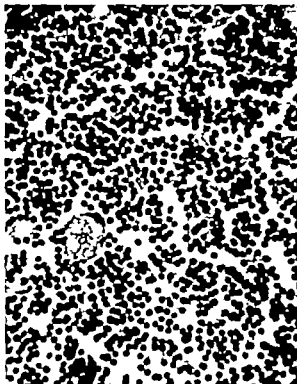


FIG. 5.—Section under higher magnification of the lymphatic node of Fig. 4, showing the lymphatic cells and dilated capillary (a). 450 approx.

in great numbers and by their influx they have simply pushed away the walls of the capillaries as well as the leucoblastic cells that may be found within them. We have found that the leucoblastic reaction of the marrow is not very pronounced in uraemic cases. In one case of uraemia there was a complete absence of any such reaction although the marrow exhibited an acute red cell engorgement and dilatation of the collapsed capillaries.

As has already been described by the writer (CHATTERJEE, 1939), there is a marked increase of eosinophils in the marrow. The average count of the eosinophils in our non-cholera cases was 3 to 6 per cent whereas the average count in our cholera bone marrows was 15 to 20 per cent. In fact, it is a common sight to see the clumps of eosinophil cells amongst the leucoblastic cells of the marrow (Fig 3).

The writer has also previously reported the presence of lymphatic nodules in the bone marrow in three cases (CHATTERJEE, 1939). The finding of these nodules was accidental and systematic search for such nodules had not been made throughout the length of the bone marrow. It may be mentioned that lymphatic hyperplasia of the intestines, particularly that of the solitary follicles, as well as enlargement of thymus, are common postmortem findings in cholera.

In another tropical disease, epidemic dropsy, we find dilatation of the capillaries. But in this condition the changes seem to affect the more well-formed sinusoids which in transverse section of the marrow appear as quadrilateral spaces at the angles of the fat cells. It is proposed to describe the latter in a separate paper.

Consequently, the following differences may be observed between the bone marrow changes of the two diseases —

Bone marrow in cholera	Bone marrow in epidemic dropsy
1 No oedema	General structure oedematous
2 Capillary dilatation affects the collapsed capillary system of DOAN as well as the sinusoids	Capillary dilatation affects more the sinusoids
3 Marked eosinophilia	No eosinophilia

#### SUMMARY

1 There is an acute dilatation and engorgement of the normally collapsed system of capillaries of the bone marrow in cholera.

2 These capillary changes are more marked than in any other organ in cholera and might at least in some way partially explain the condition of great shock in this disease.

3 Owing to the widening of the above capillaries it is possible to study their openings into the venous sinusoids of the marrow.

4 The sinusoids are also distended.

5. There is an increase in the number of eosinophils, and a variable amount of leucoblastic reaction of the marrow

6 Small lymphatic nodules have been observed in some cases.

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#### CORRIGENDA.

Vol. 40. No. 5. May 1947

N HAMILTON FAIRLEY Sidelights on malaria in man obtained by sub-inoculation experiments.

Page 653 In last line under Chart 10 *for* viable *read* variable.

Page 667 Fourth paragraph, third line, *for* VL4560 *read* VL4568.

———— Fifth paragraph, heading ( ) *for* Plasmoquine VL4568 *read* Paludrine VL4568.

———— Nine lines from bottom of pag. *for* four out of five *read* "three out of four

Page 669 Sixth paragraph, first line, *for* twenty infective bites *read* nine or ten infective bites.

## CORRESPONDENCE.

### YAWS AND SYPHILIS

To the Editor, TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene

SIR,

With reference to the discussion on Yaws and Syphilis in the TRANSACTIONS (1946) 40, 206, the following extract from HAGGARD'S *Devils, Drugs and Doctors*\* may be of interest —

"One of the earliest records of tropical medicine was made by Gonsala Fernandez de Oviedo. He was raised among the pages in the palace of King Ferdinand and Queen Isabella [of Spain], and was at Barcelona in 1493 when Columbus returned from the island of Haiti. He was intimately acquainted with most of the men who had made the voyage and many of them he knew were ill of a disease which they had contracted in America. Twenty years after Columbus's voyage, Ferdinand sent Oviedo to America as superintendent over the gold and silver mines. After a residence of twelve years, Oviedo wrote a natural history of the Spanish possessions and dedicated it to Emperor Charles V. He describes a disease known as bubas, or yaws, which he says is a very ancient disease in those localities. Oviedo identifies bubas with syphilis, for, he says, bubas is 'no other than the pocks (syphilis) which rageth and hath power over all Europe, especially among the Frenchmen. I can assure your Imperial Majesty that this disease which is new in Europe, is well known in the Antille islands lately discovered, and so very common there that almost everyone of the Spaniards who lay with the Indian women contracted it from them.' Thus it was imported from thence into Spain by those who returned with Columbus after his first or second voyage."

I am, etc ,

J WALKER TOMB

Sydney,

New South Wales

\* HAGGARD, H W (1929) *Devils, Drugs and Doctors* New York Harper pp 236, 237

### THE AETIOLOGY OF DESERT SORE

SIR,

As a fellow amateur with only a very limited acquaintance with statistical methods, one felt some hesitation in criticizing Lieut-Col S T ANNING'S recent paper in your journal (*Trans R Soc trop Med Hyg*, 40, 313). However, in the absence of more expert intervention, it is felt that the statistical treatment of his material cannot be allowed to pass without comment.

In the first place, his introduction of the idea of proportions is hardly appropriate to the particular type of experiment. It must in any case be pointed

out that, whereas Col ANNING rightly states on p. 325 that  $p$  and  $q$  in his formula represent proportions in his calculation he incorrectly substitutes absolute numbers of cases. In this particular instance the result arrived at is the same but it would be unfortunate if others copied his temptingly simple arithmetic, which might not always lead to a result so close to the correct answer. However the question at issue being whether or not cases receiving vitamin supplements heal on an average more rapidly than those without, the significance of the observed difference should surely have been tested by the formula —

$$\text{Standard error of difference of Means} = \sqrt{\frac{\sigma^2}{n} + \frac{\sigma^2}{n_2}}$$

where  $\sigma$  is the standard deviation of all observations in the two samples combined, and  $n$  and  $n_2$  are the respective numbers in the two samples (BRADFORD HILL's *Medical Statistics* p. 75).

The published data do not, unfortunately permit the application of this test, since Colonel ANNING has not thought it necessary to include a table of individual data, as recommended by BRADFORD HILL (*Ibid* p. 21) and the standard deviation cannot therefore be calculated.

A further point that calls for criticism is the inclusion, in *one* group of cases treated by several *different* therapeutic measures (viz C alone, A alone, and A and C combined).

Finally one's experience of ulcers in general would lead one to expect the time required to produce complete healing, in a group of otherwise healthy young adults, to depend on a number of factors, including at least —

- (i) The virulence of any organisms present and their sensitivity to variation in biological conditions including contact with therapeutic substances.
- (ii) The duration of the ulcer before commencement of the test, as affecting its depth and the degree of cellular reaction produced.
- (iii) The area of the ulcer at the commencement of the test, this being not entirely accounted for by (ii).
- (iv) The nature of the treatment employed.

Only after satisfying oneself that factors (i) to (iii) are equally represented in all groups can one reasonably proceed to test *statistically* the effects of (iv). The present paper presents no evidence that tests were taken to provide for such equal distribution and in view of the small samples employed it is highly unlikely that this would have established itself by chance. One is accordingly forced to the conclusion that the material in question, though interesting and suggestive, is quite unsuitable for applying statistical tests to the author's hypothesis.

I am, etc.,

J. I. LEWIS,

Medical Officer

Colonial Medical Service Nigeria.

## THE DIFFERENTIATION OF CERCARIAE AND OF THE MOLLUSCS WHICH HARBOUR THEM

SIR,

Although various experts have described in detail the characteristic features of the larvae of trematodes and claim to be able to diagnose the various species by differential staining, leading authorities in various countries still hold that it is not possible for them to give more than a tentative diagnosis until the adult worms have been reared and, even then, much uncertainty remains unless the typical eggs have been obtained from the females

The matter is important in view of attempts to produce an antigen for determining the presence of adult worms in the human subject and even for advising when efforts to destroy them can safely be discontinued. It is important because molluscan hosts of various trematode worms not uncommonly harbour one or more distinct larvae in the same individual mollusc, and it is often difficult to distinguish other forked-tailed larvae from those of *Schistosoma*

Among various species of Lymnaeidae which serve as carriers of schistosomes in South Africa, none can be regarded as more than occasional hosts except *Physopsis africana* Krauss, but further investigations are needed in regard to *Bulinus forskalii* Ehrenberg in view of its ready susceptibility to infestation with *Schistosoma haematobium* Bilharz in Mauritius, whilst *Biomphalaria* is known to convey *Schistosoma mansoni* Sambon in the sub-tropics

Among over a hundred forked-tailed larvae which have been described in South African molluscs are those of avian parasites and others whose life history has not so far been determined but are suspected of representing trematode parasites of fish, amphibians, small mammals and stock. Some of these are readily distinguished from *Schistosoma* by their eye spots but special care is needed to observe an absence of pharynx or acetabulum or varied length of the tail

Numerous bifid-tailed cercariae which I observed escaping from *Physopsis africana* Krauss at Inchanga, Natal, in 1946, seemed at first sight to be those of *Schistosoma*, but the length of the prongs of the tail prompted me to send some to the University of Strasburg and to Dr ANNIE PORTER for their identification. I was interested in a report from the former source that it would not be possible confidently to diagnose preserved cercariae

It is stated that there is no one in the world at present engaged in the study of the differentiation of the larval parasites of trematode worms though we are now in possession of valuable monographs describing those which have been studied in India, South Africa, the United States and elsewhere. From a practical point of view, more importance is necessarily attached to the working out of the respective life cycles

Much depends on a careful identification of the various molluscan hosts and, for this purpose the expert examination of the soft parts is sometimes of as great importance as that of the shell. It is not easy to believe that sinistral shells can be mistaken for dextral shells but there is good reason to fear that

this does occur and accounts for unnecessary additions to the list of molluscan hosts of some of our trematode worms.

I have observed a sinistral shell among many dextral ones in a beautiful collection of terrestrial and fresh-water snails *Bulimus forskalii* Ehrenberg being apparently confused with *Melanoides tuberculata* Müller and, when I requested some more specimens of the former mollusc from a locality in Southern Rhodesia from which I had obtained them previously could find shells only of the latter mollusc among those I received.

*Schistosoma* in South Africa is confined to non-operculated molluscs, and principally to *Physopsis* whose stouter shell enables it to withstand unfavourable environment better than many of its allies. Operculated species are more common where they are constantly exposed to great heat and periods of drought which sometimes result in the disappearance of many of the commoner intermediate hosts.

#### CONCLUSION.

Confusion in the identity of cercariae is inevitable unless the adult worms are reared and the eggs obtained.

Numerous bifid-tailed cercariae besides those of *Schistosoma* infest molluscs and more than one species may occur in the same snail. Differential staining by experts may prove an identity but authorities are still cautious about identifying preserved material. Greater caution is required in identifying individual molluscs harbouring cercariae and even in recognizing the sinistral and dextral shells.

Without the care necessary to avoid cercariae other than those of *Schistosoma*, little value can be placed on claims made for an antigen used for medical purposes.

Yours, etc.,

Durban, Natal.

F. G. CAWTON

#### A MODIFICATION OF LEISHMAN STAIN

SIR,

After trying various stains for thick and thin blood films, I have found the following modification of Leishman's stain both simple and effective in the hands of native assistants.

The dried film is stained 1 minute with full-strength stain (one to four drops). An equal amount of distilled water is added and the solution left on for 5 minutes. The stained film is then washed in tap water for 10 to 20 seconds until the bluish colour changes to a reddish purple. The film is then counterstained with ordinary Loeffler's methylene blue for 1 second, washed in tap water, dried and examined under oil immersion.

The success of this method lies in the counterstaining with methylene blue after partially decolourizing in tap water.

Yours, etc.,

Genta Mission,

Via Monrovia, Liberia, West Africa.

G. W. HARLEY

## RESIGNATION OF MISS MILDRED WENYON

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It was with profound regret that the President and Council of the Society, at their meeting of 20th March, heard of the resignation of Miss MILDRED G WENYON from the Secretaryship of the Society which she had held since 1921—nearly 26 years. During this period the Society had grown considerably, having increased its fellowship from 700 to over 1,600 and its income from a little over £600 to well over £5,000 per annum. The TRANSACTIONS had more than doubled in size.

In 1929 the Council embarked upon what was perhaps the most important enterprise of the Society's career, namely, that of procuring a home of its own in Manson House. Fellows and friends of the Society subscribed some £30,000 towards this endeavour which was brought to a successful issue in May, 1945, by the clearing of all debt incurred. These important developments and the success of the Manson House scheme were very largely due to Miss WENYON's enthusiasm and ability without which the Society would be in a very different position from that in which it finds itself today.

Many members of the Council spoke in glowing terms of the work Miss WENYON had done for the Society during her 26 years of office.

The Honorary Secretary, Prof N HAMILTON FAIRLEY, said that both he and the Joint Honorary Secretary, Brigadier J S K BOYD, had heard of Miss WENYON's decision to retire from the post of Secretary with the greatest regret. Her decision had been forced on the Council, who had no alternative but to accept it.

As regards the future, it was too much to expect any one person to continue editing the TRANSACTIONS and at the same time to be responsible for the general secretarial work of the Society. The time had come when two distinct appointments had to be made. Fortunately, Miss WENYON had agreed to continue work with the Society in a part-time capacity as Assistant Editor.

Miss WENYON's sound judgment, outstanding capabilities and drive had in the past been a dominant factor in the growth and success of the Society. She had devoted many years of her life to this objective. The present financial stability of the Society was in no small degree due to her foresight and ceaseless efforts. The fact that she remained with us as Assistant Editor of the TRANSACTIONS lessened to some extent the inevitable loss this Society must sustain by her retirement from the Secretaryship.

The Honorary Treasurer Dr O MARRIOTT said that he wished to pay tribute to the very great service Miss WENYON had always given in conducting the financial affairs of the Society.

There was no doubt that Miss WENYON during her term of Secretaryship, had saved the Society a very considerable sum of money, both directly and



indirectly. He was sure that former Treasurers would corroborate this opinion. In the carrying out of any policy Miss WENTON could always be relied on to get things done with all rapidity and efficiency.

Miss WENTON's retirement, though well earned, would be a great loss to the Society.

Dr CARMICHAEL LOW, former Honorary Secretary and Treasurer, and President from 1929 to 1933 during the early years of the Manson House scheme, said he would like to be associated with the remarks which Dr MARRIOTT had just made. No one was better able than himself to speak about the work Miss WENTON had done for the Society—she had devoted her life to it and it was no exaggeration to say that without her aid the Society never would have reached the high state it has now attained. She was most helpful in all the negotiations we had to carry through in getting Manson House, and then afterwards in running it, arranging with tenants letting the Hall and many other duties. Miss WENTON had a very fine financial brain, and all the Treasurers, including himself, had reason to be thankful she was there to guide and aid us in our duties. It was a great day and that in great part again due to her when the debt on the House was paid off giving the Society a very valuable property with no encumbrances.

He was happy to say that we were not going to lose Miss WENTON entirely as she would still continue in the service of the Society as Assistant Editor of the TRANSACTIONS, work she had so admirably done in the past. She would also he had no doubt, help with her advice in other ways.

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